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4252

Kusama's "Typhus Bacillus Immune Horse Serum" in Experimental Typhus Fever in Guinea Pigs.

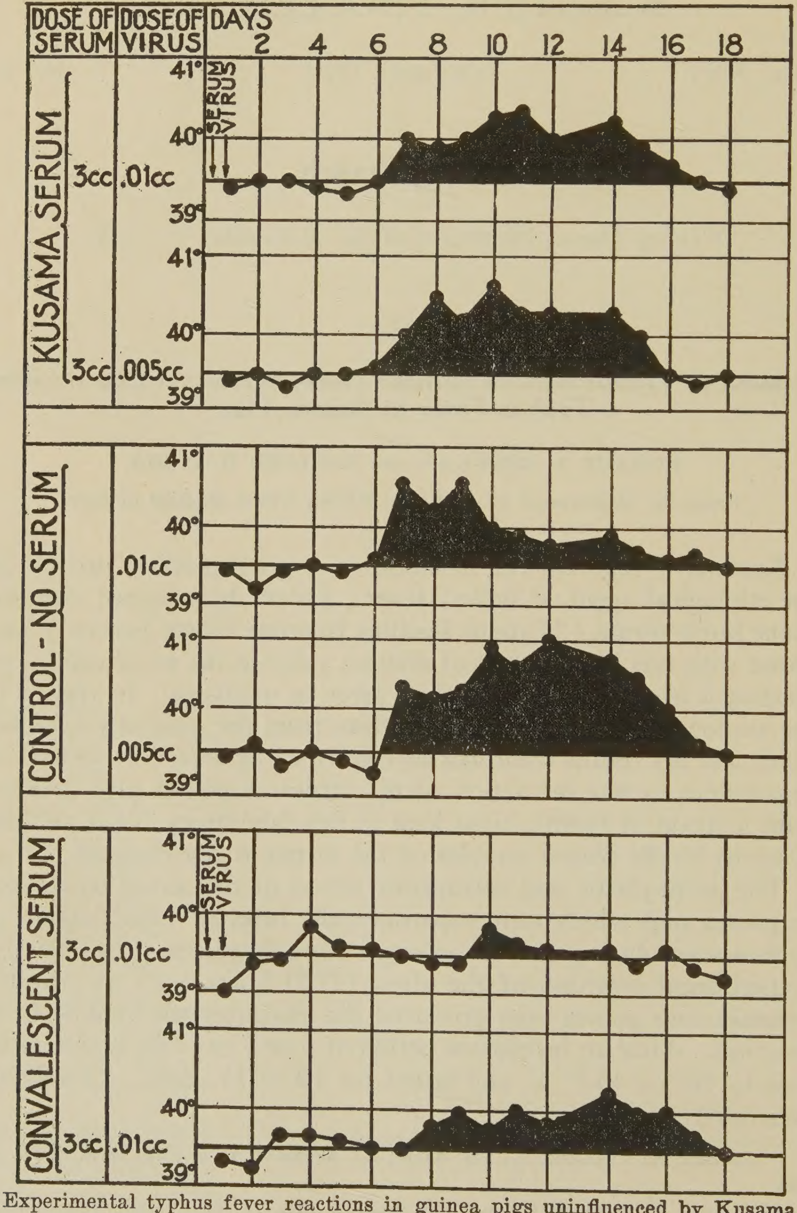
ERRATUM.

On page 193, Volume XXVI, No. 3, December, 1928, the equation

$$\frac{dx}{dt} = V_M(M_o - M_i \text{ and } V_M = \frac{1}{t} \log \frac{M_o}{M_o - M_i}$$

in footnote 5 should be transferred to line 15, after the words "we have[5]".

Prophylactic tests. 3 groups of 5 guinea pigs each were studied. One group received 3 cc. of Kusama's immune serum intraperitoneally 3 days before inoculation of typhus virus. In the second group



Experimental typhus fever reactions in guinea pigs uninfluenced by Kusama's serum compared with untreated controls and with animals partially protected by convalescent serum.

some of the animals received 3 cc. of Kusama's immune serum intraperitoneally and others the same amount subcutaneously 4 hours before injection of the virus. The last group received immune serum intraperitoneally 3 days after infection. For control tests 8 guinea pigs received virus alone while 6 were given 3 cc. of convalescent typhus serum subcutaneously 4 hours before infection. Convalescent serum is known to be of definite value in the prevention or modification of experimental typhus fever. In this experiment convalescent serum was obtained from guinea pigs on the 7th day after the temperature had returned to normal.

The guinea pigs were infected with graded amounts of brain virus ranging from 1/20 brain (0.05 cc.) to 1/200 (0.005 cc.).

The results of a typical experiment are shown in the chart. It is evident that the fever reactions of the guinea pigs which received Kusama's serum immediately before infection are about as severe and prolonged as in the control animals which were not given serum. On the other hand one of the animals injected with convalescent serum developed only a slight fever of short duration after an incubation period of 10 days. The other guinea pig showed a slight prolongation of the incubation period and a modification of the fever curve. Several guinea pigs receiving larger doses of virus (1/20 brain) were not protected by convalescent serum.

None of the animals which received Kusama's serum were immunized against infection with typhus fever virus while convalescent typhus serum appeared to have definite protective properties.

Therapeutic tests. Experiments were made in which Kusama's serum was administered daily in 3 cc. doses subcutaneously during the febrile period of experimental typhus fever in 3 guinea pigs. No beneficial effects either in the form of suppression of the fever or of shortening the febrile period were observed.

Conclusion: Kusama's "Typhus Bacillus Immune Horse Serum" had no influence on the prevention or course of experimental typhus fever in guinea pigs.

4253

Influence of Mechanical Factors on "Basal" Gastric Secretion.

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In dogs with autotransplanted gastric pouches, it has been found (in confirmation of the observations of Pavlov, Kunde and others on Pavlov-pouch dogs) that the feeding of 100-300 gm. broken bones with a cereal gruel in the evening almost invariably causes a marked secretion of gastric juice the following morning (18-24 hours later). This "hypersecretion" does not occur immediately following the gruel, but the effect of the meal persists and is enhanced 12-18 hours afterwards. Roentgenological observations have not always revealed a shadow in the stomach, but dogs killed 18-20 hours after the bone feeding always show some pieces of bones with little or no food residue in the pyloric antrum.

Neither the gruel itself nor a 200 gm. meat meal gives rise to "hypersecretion". The bones (2 cm. long) have the same effect whether raw, or boiled for 72 hours. Bone chips (less than 1 cm. long) have a much smaller and more variable effect, while finely ground, incinerated or decalcified bones are without influence. 30 gm. calcium lactate, 50 gm. calcium carbonate powder and 40 gm. marble chips (1-2 $cm.^3$) are all ineffective. Intravenous injection of calcium inhibits. The causal factor is thus due neither to organic secretagogues nor to calcium. 200 gm. of pumice stones (1-2 $cm.^3$) plus 150 gm. of meat produce a slight increase of the morning "basal" secretion.

TABLE I.

Dog		Basal Secretion mg. HCl per hr.	Meal Secretion mg. HCl per hr. (4 hrs. after meat)
A-568	18 hours after feeding gruel or meat (mean of 122 observations)	0.5	3.9
	" 200-300 gm. broken bones	19.6; 19.9; 20.9; 22.6; 40.1; 53.2;	
	" 200 chipped bones	7.5	
A-726	18 hours after feeding gruel or meat (mean of 64 observations)	0.8	6.0
	" 200-300 gm. broken bones	7.0; 20.2; 22.7; 24.0; 36.0; 38.6.	
	" 200-300 gm. ground bones	0.5; 0.7.	
	" 200 gm. pumice stones	0.4	
	" 30 gm. Ca_2CO_3 powder	0.6	
	" 40 gm. marble chips	0	

The examples in Table I indicate the increase in the basal secretion observed.

The evidence strongly suggests that "gastric hypersecretion" may arise as a result of a purely mechanical factor acting upon the pyloric humoral mechanism. Our results corroborate Savitsch's[1] work with glass beads in pyloric pouches and may lend support to his conclusion that the pyloric humoral mechanism is under nervous control.

4254

Behaviour of Denervated Spleen in Adrenalectomized Animal.

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It has already been shown that the denervated spleen contracts following exercise. Since adrenalin is one of the most powerful contractors of the spleen, it is desirable to study the behavior of the denervated spleen in animals which have been bilaterally adrenalectomized.

Dogs were used for this study. The adrenals were usually removed in 2 stages. Denervation of the spleen was performed by dividing visible nerves accompanying the splenic vessels and all intervening omental tissue between stomach and spleen. In some cases the spleen was divided into 2 and only one half denervated, the other remaining as an innervated control. In others, the upper half of the spleen was denervated without cutting the spleen into 2; the demarcation between the 2 portions was always clearcut. Immediately after operation, the denervated portion was purplish in hue and cool to the touch in contrast to the red and warm normal portion. Experiments were run before and after the 2nd adrenalectomy. The size of the spleen was traced on a soft celluloid sheet and the area calculated in cm.^{2} by means of the Amsler planimeter. The mean error of the observation is ±1.6%, so that allowance of ±5% may be made. The results summarized in the following table, are expressed in terms of the control splenic size taken as 100%.

Exercise, CO, bleeding and death bring about definite contraction of both the normal and the denervated spleen. Intravenous injection of lactic acid or sodium lactate (3-100 mg.) did not contract

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[1] Savitsch, W. W., *Russki Physiol. J.*, 1922, iv, 282.

TABLE I.

Area of innervated and denervated spleen in double adrenalectomized dogs.

Experimental condition	Remarks	% difference of splenic area	
		Innervated	Denervated
Trotting	1500 m. at 100 m. per min.	— 9.5	—13.0
,,	,, ,, ,,	— 4.6	—15.4
,,	,, ,, ,,	— 9.3	—10.4
,,	1500 m.	—15.1	—20.1
,,	2000 m.	—12.8	—13.5
Rebreathing CO-air mixture from Douglas bag	10 l. air (1% coal gas), 5 min.	+ 2.8	0.
Same	10 l. (20% coal gas), 5 min.	— 6.6	— 5.2
Rebreathing pure CO	10 l. (100% coal gas), 4 min. Fatal	—19.3	—10.1
Rebreathing CO-air mixture	10 l. air (30% coal gas), 4 min.	—36.4	— 1.0
Rebreathing pure O_2	10 l. pure O_2, 5 min.	+ 0.2	+ 0.6
Rebreathing pure CO	10 l. (100% coal gas), 3 min. Fatal	—25.9	— 4.8
Bleeding	100 cc. blood	—	—9.6, —8
Death (after CO)	4 min. post-mortem	—29.9	— 6.1
,, ,,	3 min. post-mortem	—28.5	—19.9
CO_2	3-5 min.	— 7.3	+ 0.9
Stream of CO_2 to nostrils	3-5 min.	— 0.5	— 2.9
$C_3H_6O_3$ neutralized with NaOH	3 mg.	0.4	— 1.9
	7 mg.	—15.8	+ 2.2
$C_3H_6O_3$	50 mg.	—12.6	— 7.8
	50 mg.	—12.0	+ 1.3
	100 mg.	+ 2.5	— 0.5

the spleen, but tended rather to increase its volume. CO_2 and O_2 inhalation for 3-5 minutes have practically no effect.

The contraction of the denervated spleen in doubly adrenalectomized animals may be accounted for either by a local postural change in the splenic vessels or musculature in response to changing volume flow (diminished splanchnic volume flow in exercise and hemorrhage) or to a local effect of anoxemia (CO and post-mortem effect?).

4255

Influence of "Chilies" (Capsicum Annum, L.) on Digestive Functions and Metabolism.

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The use of chilies or Capsicum as a condiment by tropical and sub-tropical peoples (*e. g.*, in "curry" in Polynesia, Malaysia, India, Arabia, tropical Africa; in "chili-con-carne" in Central America; "lah-ti shou" (hot foods) in southern China) has led us to inquire into the reason for such a widespread habit. We have, therefore, determined the influence of *Capsicum annum, L,* on the digestive functions and basal metabolism. *C. annum, L,* var. gr. Sendt., was employed in the gastric secretion observations on man and in one metabolism experiment.

Salivary secretion. Capsicum produces a copious flow of saliva, both in dog and man. In man 1 cc. of Capsicum extract prepared by grinding 10 gm. fresh Capsicum with 20 cc. distilled water and straining through muslin, was introduced into the mouth, quickly distributed by the tongue and spat out. The mouth was then closed for 5 minutes, at the end of which time the accumulated saliva was collected, measured and compared with the amount obtained during a control period without Capsicum. In the unanesthetized dog the Capsicum extract was injected into the mouth and the saliva collected from fistulae of the submaxillary and parotid ducts.

TABLE I.

Subject		Procedure	Salivary Flow
			cc. per 5 min.
Man		Control	3.5
		Capsicum (1 cc.)	14.8
Dog	Partoid	Control (1 cc. 0.4% HCl)	0.
		Capsicum (0.2 cc.)	1.0 (15 drops)
	Submaxillary	Control (1 cc. 0.4% HCl)	0.1 (2 drops)
		Capsicum (0.2 cc.)	6.5 (50 drops)

Gastric secretion. For the study of gastric secretion in dogs, Heidenhain- and Pavlov-pouch animals were used. Freshly minced Capsicum was given by mouth (*i. e.,* swallowed), sometimes in gelatine capsules and sometimes without, in amounts ranging from 4 to 20 gm. The following is typical of 23 observations:

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[*] Rockefeller Foundation Fellow.

TABLE II.

Time	Procedure	Gastric juice	Free HCl	Total HCl
		cc.	mg. %	mg. %
9-10	Control	0.2	0	—
10-11		0.1	0	—
11	10 gm. Capsicum			
11-12		1.5	0	—
12- 1		1.9	0	—
1-2		1.3	0	—
2-3		1.3	0	—
3-4		1.0	0	—
4	200 gm. meat, 100 cc. water			
4-5		14.0	316	381
5-6		7.6	332	421

The most usual effect of Capsicum upon the stomach appeared to be a slight increase in mucous secretion. It seldom affected the acidity of the gastric juice. Whether with, before or after meals, it neither inhibited nor augmented the usual gastric response.

Experiment with a Rehfuss tube on man showed that the rinsing of the mouth with Capsicum (1 cc.) had no significant secretory (psychic) effect in the subject tested, but that the introduction of Capsicum (20 cc.) into the stomach through the stomach tube caused a distinct response, as shown in the following example. It is of interest to note that on taking Capsicum a warm, tingling sensation is perceived in the epigastrium.

TABLE III.

Time	Procedure	Gastric juice	Free HCl	Total HCl
		cc.	mg. %	mg. %
9:00- 9:15	Control	7.0	0	64.8
9:15- 9:30	”	5.0	0	72.9
9:30- 9:45	”	3.5	0	79.9
9:45-10:00	1 cc. H_2O in mouth	3.0	0	72.9
10:00-10:15	1 cc. Capsicum in mouth	4.0	0	81.0
10:15-10:35	20 cc. H_2O into stomach tube	9.0	0	40.5
10:35-10:55	20 cc. Capsicum into stomach tube	11.7	170	219.0

Pancreatic and biliary secretion. A very slight and transient inhibitory effect on these secretions was obtained on introducing Capsicum into the duodenum of the etherized dog.

Gastric motility. Experiments by the balloon method in dogs with gastric fistulae showed that Capsicum sometimes caused a slight augmentation of tone, but the effect was transient and not constant. Roentgenological observations showed that meals with and without Capsicum are emptied in about the same time.

TABLE IV.

Nature of Meal	Gastric Emptying Time	
	hr.	min.
300 gm. corn-bean flour, 50 gm. barium,	4	30-36
Ditto + 20 gm. minced Capsicum,	4	15
200 gm. meat, 100 cc. water, 50 gm. barium,	7	25
Ditto + 20 gm. minced Capsicum,	7	35

Bowel movement. Excessive amounts (above 20 gm.) of Capsicum may induce frequent bowel movements, as reported by two of the subjects in the metabolic experiments.

Specific dynamic action. 2 of the 4 individuals tested (Benedict-Knipping method) were well accustomed to eat peppery food. No effect on metabolism was obtained even with the ingestion of 100 gm. fresh Capsicum. The data are presented in the following table:

TABLE V.

Subject	Time	Remarks	R. Q.	Calories per 24 hrs.
C. Y. L.	8:00	Control	0.70	1505
	8:12	63 gm. Capsicum (Var. gr. Sendt.)		
	9:40		0.74	1323
	10:15		0.77	1459
C. Y. L.		Control	0.84	1373
	8:35	100 gm. Capsicum		
	9:00		0.72	1380
	10:00		0.87	1378
P. K. K.	7:55	Control	0.73	1331
	8:30	27 gm. Capsicum followed by 100 cc. water		
	9:30		0.80	1362
	10:30		0.76	1333
Y. K. Y.	8:45	Control	0.78	1592
	9:15	"	0.76	1523
	9:40	45 gm. Capsicum followed by 300 cc. water		
	10:40		0.73	1438
	11:40		0.78	1415
T. Y. L.	9:00	Control	0.73	1393
	9:40	About 10 gm. Capsicum with 300 cc. water		
	9:45		0.76	1379
	11:00		0.75	1475

Conclusions. With the exception of salivary and possibly the human gastric secretion Capsicum in moderate amounts has no significant effect on the digestive functions. It does not affect the metabolism. As its irritant property increases the salivary flow, it no doubt facilitates the swallowing and digestion of dry, starchy food, *e. g.*, cooked rice. In people who take Capsicum regularly, a conditioned reflex may be induced and thus give rise to gastric secretion.

It has been suggested that Capsicum is taken because of its cooling action due to perspiration (chiefly cephalic), but this certainly cannot be true for all persons. One of my subjects in the metabolic experiment ingested 100 gm. fresh Capsicum, yet did not sweat. Probably the main reason for its being widely used and liked is that it stimulates appetite, more especially as a hot climate tends to produce anorexia.

4256

Growth and Reproduction of Rats on Vitamin C Free Diet.

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Harden and Zilva[1] and Drummond[2] believed that albino rats could not fully dispense with the antiscorbutic vitamin without restriction of their normal development. Osborne and Mendel[3] were unable to accept this conclusion since it had not been ruled out that the delayed development demonstrated by the first named authors was due to a vitamin B deficiency of their yeast preparation. Neither could it be ruled out that the growth-promoting effect of the fruit juices added was due to a more ample supply of vitamin B rather than to the vitamin C content of the juices.

Parsons[4] made the important observation that rats which had been fed on a practically vitamin C free diet for as long as 213 to 247 days showed considerable amounts of this vitamin in their livers. This, as McCollum[5] points out, constitutes conclusive evidence of synthesis of vitamin C by this species. Parson's experiment, however, does not directly answer the question whether better growth and nutrition could be secured by the addition of an antiscorbutic substance to the diet of albino rats.

The experiments here to be reported show that the addition of the antiscorbutic vitamin had no growth-promoting effect when given to white rats which for 2 generations or more had been reared and raised on a vitamin C free diet. The experiments were carried out on the third, fourth, and fifth generations. One group of the animals of the third generation, which did not receive any addition of vitamin C, was used as controls.

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[1] Harden, A., and Zilva, S. S., *Biochem. J.*, 1918, xii, 408.

[2] Drummond, J. C., *Biochem. J.*, 1919, xiii, 77.

[3] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1920, xli, 554.

[4] Parsons, H. T., *J. Biol. Chem.*, 1920, xliv, 587.

[5] McCollum, E. V., "The Newer Knowledge of Nutrition," 2nd ed., 1923.

The experiment has extended almost 20 months. The basal diet was composed of: Soybean meal 35, mung bean meal 30, millet flour 30, NaCl 1, $CaCO_3$ 1.5 and cod liver oil 2. These ingredients, mixed with boiling water, were cooked in a double boiler for 20 to 30 min. This diet invariably produced scurvy in guinea pigs and caused death in less than 4 weeks. As the source of vitamin C fresh cabbage (Peking variety) was used in amounts of 2 gm. per rat each day. The same amount fed to guinea pigs prevented scurvy indefinitely and supported normal growth.

At the age of 8 months the third generation rats on cabbage feeding gave the following body weights in gm.: 282, 334, 360, 340 (males), 264, 272, 248, 230 and 290 (females); those on basal diet alone: 258, 346, 330, 322 (males), 262, 216, 244, 218 and 230 (females). The cabbage ration rats successfully reared 3 out of 4 litters; those on the basal diet, 2 out of 4 litters. The 4 animals of the fourth generation on cabbage feeding at the age of 18 weeks weighed 192, 188 gm. (males), 170 and 164 gm. (females). Two litters were cast but not reared. The 4 rats of the fourth generation on basal diet at the age of 17 weeks and 5 days gave the following body weights in gm.: 206, 254 (males), 158 and 172 (females). One small litter constituting the fifth generation was successfully weaned at the age of 23 days.

4257

New Technique for Feeding Sandflies (Phlebotomus) for Experimental Transmission of Kala-Azar.

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In the experimental attempts to transmit kala-azar by the bites of sandflies, it has been found desirable to substitute an artificial feeding on blood rich in Leishmann-Donovan bodies for the natural feeding upon infected animals, in order to obtain an abundant growth of flagellates within the flies. This blood is obtained by mixing the blood of a normal animal with the crushed spleen of a hamster heavily infected with kala-azar.

For such artificial feeding we used the technique of Hertig and Hertig.[1] This technique was learned in a few days and in the course of 2 months during the summer of 1928, 565 flies were fed. During this work it became clear that this technique had a number of limitations. The flies had to be fed one by one, making the procedure tedious. During the etherization and handling of the flies, many were injured. A satisfactory engorgement was obtained with *P. sergenti,* while *P. major* only in rare instances could be fully engorged. In order to feed a larger number of flies with a minimum of injury and a maximum of engorgement, the following technique has been devised.

Technique. Etherize a hamster heavily infected with kala-azar, pin its feet on a wooden board, and place its head and neck between the arms of a U-shaped wooden block. Remove the hair over the anterior surface of the neck and chest by pulling and sterilize the skin with alcohol and ether. With aseptic precautions, make a median incision through the skin extending from the anterior end of the lower jaw to the middle of the sternum. Pull apart the skin flaps and pin them to the arms of the U-shaped block, the upper surface of which is slightly raised above the level of the head and neck. Cut the carotid artery on one side and, as the blood wells up, quickly draw it into a sterile pipette. Practically all the blood that is obtained by bleeding can be drawn into the pipette. Defibrinate and centrifuge the blood.

Remove the spleen aseptically, cut the whole or a part of it in small pieces, and place it in a small test-tube 3 inches long and 1/3 inch in diameter. Crush it thoroughly with a glass rod. Into this crushed material stir the blood plasma and centrifuge the mixture to throw the cell clumps to the bottom. Add enough red cells to the supernatant fluid to give the resultant mixture the approximate concentration of normal blood.

Place a piece of flannel in the shallow dish on the upper surface of an aluminum feeding stage (Figs. 1 and 2B) and add the blood-parasite mixture. In order thoroughly to saturate the flannel piece, work the mixture into the flannel by repeated pressing. This flannel represents the subcutaneous tissue of the animal. Cover with a piece of skin from the hamster, removed during the centrifuging of the spleen-plasma mixture. The skin most suitable for this purpose is that of the back. Before applying the skin to the stage, remove the hair on the external surface and also the thin layer of muscle attached to the inside. Do not sterilize the external skin surface, as the flies do not bite as well on sterilized skin. Place the

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[1] Hertig, Arthur T., and Hertig, Marshall, 1927, lxv, 328.

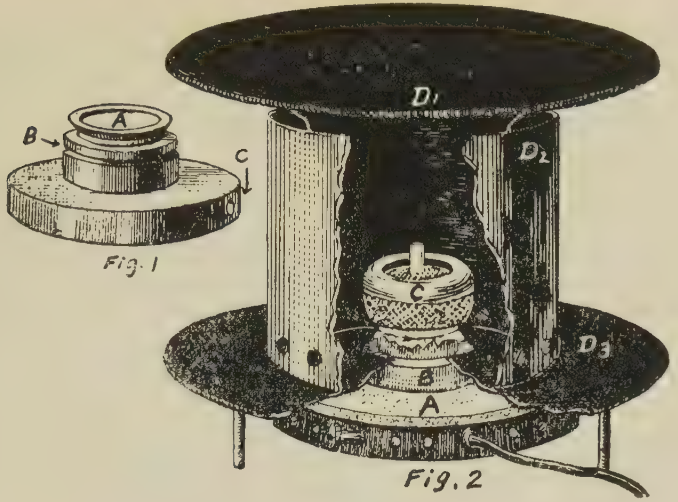

FIG. 1. *Feeding Stage.*

(A) Dish, about ¾ inch in diameter, for the holding of flannel. (B) the groove for the tying of the skin which covers the flannel. (C) a hole for the insertion of a thermometer to register the temperature of the feeding stage.

FIG. 2. *Feeding Outfit.*

(A) The electric heater (this heater is manufactured by Chicago Surgical and Electrical Co.) (B) the feeding stage covered with hamster skin. (C) the cage containing flies. (D1), (D2), (D3) 3 separate parts of cover, all painted black.

stage thus prepared on an electric heater (Fig. 2A) at a steady temperature of 35° C., and put on the skin surface a cage (Fig. 2C) containing the flies hatched in the laboratory during the last 24 hours. Arrange a set of covers (Fig. 2, D1, D2, D3) to keep the cage dark and to prevent the rising of heat from the heater.

Many flies may be put into one cage for feeding, but the best result is obtained when the number is not too large. In 30 minutes, 95% of the flies may be fully engorged if the number of flies in the cage does not exceed 20, whereas the percentage drops to 50 or 60 when the number of flies is 60 or more. There are always a number of flies which will not feed until they are 24 hours old. The feeding is best done in the evening, but is sometimes successful in day time, if the covers are properly set.

Flies fed by this method almost never die within the first 3 or 4 days of life. During the months of July and August, with an average temperature in the laboratory of 27° C. and a humidity of approximately 60%, the average span of life of these flies, unless refed on the fourth or fifth day, was 5 or 6 days. In order to lengthen this period to give the parasites longer time to develop, the flies were kept in an ice box at a temperature of approximately 21°

C. With refeeding some of the flies at this temperature lived for 18 days, and an abundant growth of flagellates in the flies were obtained.

For breeding purposes flies are fed on the whole blood of a normal hamster, on the same stage.

From July 16th to August 31st, 1928, 2131 female sandflies (1918 *Phlebotomus major* and 213 *Phlebotomus sergenti*), practically all that were hatched in our laboratory. were fed so satisfactorily that we feel justified in suggesting the adoption of this technique for the artificial feeding of sandflies. The method described is an attempt to imitate the natural conditions under which the flies feed, but the artificial conditions are better than the direct feeding on animals. The immobility of the feeding stage is a decided advantage, as the biting insects are frequently disturbed by the movements of the live animal. The ease with which the insect gets blood, practically every time the stylets pierce the skin, is another distinct advantage of this feeding stage.

The principle of this method might be used in experiments on transmission of diseases in which biting insects play a rôle. The skin preparation of the feeding stage might serve either as the source of infection, or as a medium for transmission.[2]

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[2] Hu, C. H., Huie, Dorothy, and Lee, C. U., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi.

4258

Slapping as a Factor in Transmission of Kala-Azar by Sandflies (Phlebotomus).

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The results in the experimental transmission of kala-azar thus far have been uniformly negative.[1, 2] Shortt, attempting transmission by means of sandflies, attributes his negative results to insufficient susceptibility of the experimental animals rather than to inability of the fly to expel the flagellates from its buccal cavity into the wound while feeding. We are inclined to believe that the negative results

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[1] Shortt, H. E., Barraud, P. J., and Craighead, A. C., *Indian J. Med. Res.*, 1927, xiv, 589.

[2] Young, C. W., and Hertig, Marshall, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 823.

might have been due to the overlooking of one natural factor not previously considered, namely, the crushing of the fly by slapping the itching skin, where the fly is feeding. To ascertain the importance of this factor in the transmission of kala-azar, a method has been worked out, imitating as far as possible the natural conditions under which the flies feed and are crushed.

The sandflies, *P. major,* are fed with the blood-parasite mixture on the feeding stage within 24 hours after hatching,[3] and are then kept in the ice box at 21° C. After 6 to 10 days, they are allowed to refeed and, while feeding, are crushed by slapping. This is occasionally done as late as the 18th day, if the flies have had a second feeding.

1. *The feeding stage.* This stage is like the one for the blood-parasite mixture, except that the dish is only ½ inch in diameter. The flannel is cut a little smaller than the dish and is cut in 2 equal halves. It is then pressed and saturated with defibrinated rabbit blood, and covered with a piece of skin from a normal hamster. Strictest asepsis is necessary in these preparations, but the heavily contaminated external surface of the skin is not sterilized for reasons which are given below. The prepared feeding stage is placed on the electric heater set at 35° C., and the slapper is adjusted to the skin surface.

2. *Feeding and slapping.* After the slapper is set with the lower end of the piston lifted by the magnet to a level ½ inch above the skin surface, a fly is introduced through the side-tube which is then plugged with cotton. With a bright electric light the fly is closely watched. When its abdomen is being engorged the switch for the electric magnet is turned off, the piston instantly drops, and crushes the fly while its biting parts are still in the skin. The fly is then immediately fixed for serial sections. The skin is removed from the stage and each of the two pieces of flannel is put into a separate tube of NNN media for the cultivation of flagellates. The cultures are examined at the end of 3 weeks. (Table I.)

The process described is time-consuming, for the flies must be fed and slapped one by one. Most of them do not feed, probably due to the sudden change in surroundings, and to the presence of a bright light. Those which do feed are not as fully engorged as when fed in the dark, usually stopping to suck when they show blood in the thorax or in the anterior abdomen.

In a second series of experiments the flies were allowed to withdraw their stylets after feeding without being slapped, after which the flannels bitten by them were cultured. (See Table II.)

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[3] Hu, C. H., and Lee, C. U., **PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi.**

TABLE I.

A record of the flies slapped in the act of feeding and the result of cultures made from the flannels.

Culture No.	Date of hatching and first feeding	Interval between 1st feeding and slapping	Flagellates in serial sections	Culture of flannel		Remarks
				A.	B.	
15	Aug. 18	9 days	+	—	—	
16	" 18	9 "	+	—	—	
17	" 19	8 "	+	—	Cont.	
18	" 19	8 "	+	—	—	
20	" 18	10 "	+	—	—	
22	" 19	10 "	+	—	—	
23	" 19	10 "	+	—	Cont.	
24	" 17	12 "	+	—	"	
26	" 22	8 "	+	—	"	
28	" 25	6 "	+	—	+	
29	" 14	18 "	+	—	Cont.	Refed with rabbit blood on August 22.
30	" 18	14 "	+	—	—	Refed with rabbit blood on August 27.
32	" 26	7 "	+	—	—	
33	" 26	7 "	—	—	—	
34	" 27	7 "	+	—	—	
36	" 28	7 "	+	—	—	
37	" 28	7 "	—	—	Cont.	
39	" 29	7 "	+	—	—	
39a	" 29	7 "	+	—	—	
40	" 30	7 "	+	—	—	
41	" 30	7 "	+	—	—	
42	" 30	7 "	+	—	Cont.	
43	" 30	7 "	+	—	—	
44	" 31	8 "	+	—	—	
46	Sept. 2	8 "	+	—	—	
47	" 6	7 "	—	—	—	
48	" 6	7 "	+	—	—	

Cont. = Contamination by bacteria.

TABLE II.

A record of the flies not slapped and the results of cultures of the flannels.

Culture No.	Date of hatching and first feeding	Interval between first feeding and slapping	Date of death of fly	Flagellates in serial sections	Culture of flannel	
					A.	B.
19	Aug. 14	8 days	Aug. 27	+	—	—
27	" 22	9 "	" 31	+	—	—
35a	" 27	7 "	Sept. 8?	+	—	—
38	" 28	7 "	" 8	+	—	—
38a	" 28	7 "	" 8	—	—	—
38b	" 28	7 "	" 8	—	—	—
44	" 31	8 "	" 10	+	—	—
45	" 31	8 "	" 12	+	—	Cont.
49	Sept. 6	7 "	" 13	+	—	—

Cont. = Contaminated by bacteria.

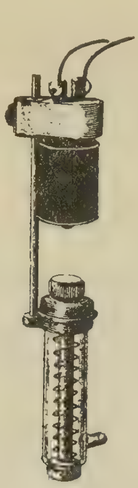

FIG. 1. *The Slapper.*

The upper end of the copper piston is screwed to an iron cap (A), the lower end is expanded, a little less than 3/8 inch in diameter, and projects 3/16 inch below the bottom of the glass tube. The glass tube is 2.5 inches long and 3/8 inch in its inner diameter. 1/4 inch above the lower edge of the tube is a short side-tube for the introduction of the fly. Around the piston is a steel-wire spring which pushes down when the current in the magnet (B) is broken.

Table I shows one positive experiment out of 27. Here a positive, non-contaminated culture of flagellates morphologically typical of Leishmann-Donovan bodies was obtained from one of the 2 flannel pieces on the feeding stage. Of the remaining 26 flies 23 contained flagellates, but failed to infect the flannel. Seven flannel pieces showed contamination by bacteria. Table II shows 9 experiments without slapping, all negative. Two of the 9 flies proved microscopically negative for flagellates. One flannel piece was contaminated by bacteria. All contaminations were due to direct introduction of bacteria through the hamster skin by the stylets. This statement is based upon the fact that in every case only one of the 2 flannel pieces was contaminated.

From the one positive culture (Table I) subcultures were made. We believe this positive finding to be significant, as demonstrating a means through which transmission of kala-azar might take place. As to the minute mechanism in play in our experiment we believe that the slapping, when undertaken at the right moment, causes a back flow of blood through the stylet all the way from the midgut. During the feeding there certainly are moments of relaxation of the crinkly posterior portion of the pharynx, making such a back flow possible.

Slapping does occur in nature as all know. If slapping is a necessary factor, explanation for the apparent high immunity to kala-azar among infants might be found in as simple a fact as their inability to slap when bitten.

4259

Antipneumococcus Protective Substances in Normal Pig Serum.

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From the Department of Medicine, Peking Union Medical College.

It has been generally held that natural immunity to the Pneumococcus, in common with other pathogenic micro-organisms, depends on a fundamental cellular difference of the host rather than on circulating antibodies.[1, 2] Bull and McKee,[3] however, showed that normal chicken serum was capable of protecting mice and guinea pigs against many times the fatal dose of virulent pneumococci. Furthermore, in a recent publication,[4] evidence was presented to indicate that a high concentration of antipneumococcus opsonins in the blood of naturally resistant mammals forms a common means of defense against pneumococcus infection. These findings would suggest that humoral defensive elements may play a much more important rôle in the mechanism of natural immunity than has hitherto been believed. This report presents further evidence in support of this view.

In the present study, serum from pigs, a naturally pneumococcus-resistant species, has been employed. Table I represents the protocol of an experiment in which white mice weighing about 20 gm. were each given intraperitoneal injections of 1 cc. of pig serum and 4 hours later varying amounts of an actively growing culture of a virulent Type I pneumococcus. Sera from susceptible animals, the rabbit and the guinea pig, were used as controls. It is clearly shown that normal pig serum protected mice against 10,000 times the minimum fatal dose while rabbit or guinea pig serum conferred no protection on mice against pneumococcus infection. Mice receiving less than 0.01 cc. of culture were protected by the pig serum, whereas 0.000,000,1 cc. of the same culture alone killed mice regularly.

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[1] Zinsser, H., "Infection and Resistance," New York, 3rd edition, 1923, 68.

[2] Eastwood, A., *Rep. Pub. Health and Med. Subj.*, Ministry of Health, No. 22, 1923, 15.

[3] Bull, C. G., and McKee, C. M., *Am. J. Hyg.*, 1921, i, 284.

[4] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1927, xlvi, 239.

TABLE I.

Protective Action of Pig Serum Compared with Rabbit and Guinea Pig Sera for White Mice.

Kind of Serum	Amount of serum	Amount of pneumococcus culture	Result
	cc.	cc	
Pig serum	1.0	0.01	D
	1.0	0.001	S
	1.0	0.0001*	S
Controls with sera of susceptible animals			
Rabbit serum	1.0	0.0000001	D
Guinea pig serum	1.0	0.0000001	D
Controls without serum	—	0.000001	D
	—	0.0000001	D

D, died in 12 to 48 hrs. S, survived 7 days.

* Mice receiving pig serum and 0.00001, 0.000001 and 0.0000001 cc. pneumococcus cultures also survived.

Similarly, pig serum has been found to protect mice against virulent Type II and to a lesser degree Type III pneumococci.

In two previous studies,[5, 6] results were recorded to suggest the specificity of natural pneumococcus-opsonins. Attempts, therefore, have also been made to determine if the antipneumococcus protective substances in normal pig serum were type specific. For this purpose, pig serum was first absorbed by a Type I pneumococcus, and the absorbed serum after centrifugation and Berkefeld filtration was employed in mouse protection tests against a Type I and Type II pneumococci. Results of such an experiment are given in Table

TABLE II.

Pig Serum Absorbed by Pneumococcus Type I.

Pig Serum	Amount of serum	Amount of Type I pneumococcus cult.	Amt. of Type II pneumococcus cult.	Result
	cc.	cc.	cc.	
Absorbed	1.0	0.000001		D
	1.0	0.0000001		D
	1.0		0.1	D
	1.0		0.01	S
	1.0		0.001	S
Unabsorbed	1.0	0.01		D
	1.0	0.001		S
	1.0	0.0001		S
	1.0		0.1	D
	1.0		0.01	S
	1.0		0.001	S
Controls without serum	—	0.0000001		D
	—		0.0000001	D

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[5] Sia, R. H. P., *J. Exp. Med.*, 1926, xliii, 633.

[6] Sia, R. H. P., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 709.

II. It is seen that while the absorbed serum lost all of the protective substances against Type I pneumococcus, the protective properties for the Type II pneumococcus remained unaltered.

Specific absorption with a Pneumococcus Type II has given equally convincing results.

Summary. By means of mouse protection tests, it has been possible to demonstrate the existence of antipneumococcus protective substances in normal pig serum. Furthermore, these protective substances have been found to be type specific.

Pacific Coast Branch.

Stanford University Medical School, December 12, 1928.

4260

Effect of Hyperpyrexia, Produced by Baths, on Permeability of the Meninges.

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It has been previously demonstrated that after sodium bromide is given by mouth for 5 days, a comparison of the bromide found in the blood and spinal fluid shows about 3 times as much bromide in the blood as in the spinal fluid. In certain pathological states of the nervous system this ratio shows a characteristic change. It was concluded that this method gives us a reliable method of estimating changes in permeability.

We used Malamud's[1] modification of Walter's[2] technique on patients whose mouth temperatures had been raised to 103°-106° F. for at least 30 minutes daily for at least 5 consecutive days. The patients were put into an ordinary bath tub or, more comfortably, into a continuous bath tub, the temperature of the bath being gradually raised to 110° F.

The following examples will illustrate the range of the permeability quotient.

TABLE I.

	P. Q.	Consecutive Days Fever	Total Hours Fever	Mouth Temperature
No. 171712				
Control	3.0			
	1.95	7	3	103°-105°
	1.7	12	6	103°-105°
	1.6	25	13	103°-106°
No. 180857				
Control	3.0			
	3.0	3	1	103°-105°
	1.8	9	2	103°-105°
	1.8	16	4	103°-105°

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[1] Malamud *et al.*, *Arch. Neurol. and Psychiat.*, 1928, xx, 780.

[2] Walter, F. K., *Z. f. d. ges Neurol. u Psychiat.*, 1925, xcv, 522.

Stecker, H. A., *J. Mental Sci.*, 1928, lxxiv, 73.

Conclusions: The ratio of bromide in blood and spinal fluid is changed after fever. This hyperpyrexia must be greater than 103° F., continued at least 30 minutes and be repeated at least 5 successive days. The permeability quotient (P. Q.) in all cases was lowered, showing increased permeability. The maximum lowering was a P. Q. of 1.6; the least result was 2.7. A lower temperature than 103° F. or one continued for less than 5 days produced uncertain results.

The above results indicate that a hyperpyrexia produced by hot baths is capable of lowering the permeability quotient in the same manner as does the malarial fever.

4261

Presence of Afferent Nerve Fibers in Meningeal Blood Vessel Walls.

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The presence of vasomotor nerves in the cerebral blood vessels seems well established as a result of the work of Nothnagel,[1] Wiggers,[2] Brown,[3] Anrep and Starling,[4] and Forbes and Wolff.[5] Anatomical studies, particularly by Alexander,[6] Gulland,[7] Huber,[8] Hunter,[9] Traum,[10] and Stohr,[11] have suggested the presence of sensory nerve-endings in the pial blood vessel walls. We have been unable to find any physiological work on these supposed afferent nerve-endings.

In connection with a series of dogs, in which dilatation of the pial blood vessels was studied, as previously reported,[12] we made a number of observations on reflex stimulation of respiration and blood pressure following electrical stimuli applied to the meningeal vessels and directly to the dura. As controls, we used the same strength of faradic current to obtain reflex effects from the stimulation of the femoral blood vessels. We varied the procedure considerably in order to avoid unfair conclusions with regard to the relative sensitivity of these various areas. In these experiments the depth of anesthesia must be maintained the same throughout. Furthermore, we noted that pressure variations in the application of the electrodes would cause variations in the reactions, and that, under some circumstances, cauterization might occur. Great care was necessary to avoid these complications. We considered as significant, changes beyond 8 per minute in respiratory or heart rate, and greater than 6 mm. of mercury in the mean blood pressure.

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[1] Nothnagel, H., *Virchow's Arch.*, 1867, xl, 203.

[2] Wiggers, C. J., *Am. J. Physiol.*, 1907, xx, 206.

[3] Brown, E. D., *J. Pharm. Ex. Therap.*, 1916, xiii, 185.

[4] Anrep, G. V., and Starling, E. H., *Proc. Roy. Soc. Biol. Sci.*, 1924-25, xcvii, 463.

[5] Forbes, H. S., and Wolff, H. G., *Arch. Neurol. Psych.*, 1928, xix, 1057.

[6] Alexander, W. T., *Arch. f. Mikrosc. Anat.*, 1875, xi, 231.

[7] Gulland, L., *Brit. Med. J.*, 1898, ii, 781.

[8] Huber, C. C., *J. Comp. Neur.*, 1899, ix, 1.

[9] Hunter, W., *J. Phys.*, 1900-01, xxvi, 465.

[10] Traum, E., *Zeit. f. Anat. u. Entwick.*, 1925, lxxvii, 488.

[11] Stohr, P., *Zeit. f. Anat. u. Entwick.*, 1922, lxiv, 555.

We found that electrical stimulation applied to the blood vessels of the dura mater or pia mater caused a greater effect than the same strength of current applied directly to the meningeal surfaces between blood-vessels. Surgeons operating on the brain under local anesthesia, but without the meninges anesthetized, have noted in cutting the meninges that pain is felt chiefly when the meningeal vessels are cut, or traumatized.[13]

Stimulation of the pial and dural vessels usually caused a significant decrease in respiratory rate. Stimulation of the femoral vessels, however, usually resulted in a significant fall in blood pressure without any marked effect on the pulse rate. There was decreased sensitivity to the electrical stimulation in all the vessels tested, the longer the experiment was continued.

The results were not clear-cut enough to be definitely conclusive but they are worth reporting in the effort to call attention to the need of further experimentation in this field. The presence of sensory nerve fibers in the meningeal blood vessels offers an explanation of headaches occurring with presumed pressure changes in the cerebral blood vessels.

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[12] Leake, C. D., Hitz, J. B., and Kammer, A. G., *J. Pharm. Ex. Therap.*, in press; Leake, C. D., Loevenhart, A. S., and Muehlberger, C. W., *J. Am. Med. Assn.*, 1927, lxxxviii, 1076.

[13] Independent personal communications from Dr. A. S. Crawford and Dr. H. Cushing. Dr. H. Naffzigger agrees with this observation only with reservations.

4262

Factors Affecting Penetration of Methylene Blue and Trimethyl Thionine into Living Cells.*

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Since some confusion has arisen lately concerning the identity of the dye which penetrates into the vacuole of living plants of *Nitella* and *Valonia,* the writer has undertaken to analyze the causes of this apparent discrepancy.

Irwin[1, 2] has attempted to show by spectrophotometric determinations that the dye from solutions of methylene blue which penetrates the sap of these plants, is not methylene blue as originally stated by the writer[3, 4] but is rather some lower oxidation product, trimethyl thionine. This forms readily in alkaline solutions of methylene blue and penetrates as a free base, whereas methylene blue is completely dissociated and according to Irwin does not penetrate living cells except during injury.

The writer, on the other hand, also using the spectrophotometer to determine the identity of the dye found in the sap of these plants, found only methylene blue present.

An analysis of the differences in methods used by Irwin and the writer has been made. The following main points of divergence have been tested.

1. *pH value.* The writer used pH 8.83 while Irwin used 9.5 and 10.9. The use of the higher pH values in the external solution introduces 2 errors: first, precipitation of Mg (at pH 10.9) from sea water which produces a solution unbalanced with respect to cations. Such unbalanced solutions affect the permeability of all plant cells for which we have any data. The second effect of high pH value is the relatively rapid conversion of methylene blue into trimethyl thionine and related dyestuffs.

2. *Impurity of the dye.* The writer used Clark's methylene blue

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* The expenses of this investigation were partly defrayed from the Sigma Xi Fellowship Fund and the Bache Fund of the National Academy of Science.

[1] Irwin, M., *J. Gen. Physiol.*, 1927, x, 927.

[2] Irwin, M., *J. Gen. Physiol.*, 1928, xii, 147; PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 563.

[3] Brooks, M. M., *Am. J. Physiol.*, 1926, lxxvi, 360.

[4] Brooks, M. M., *Univ. of Calif. Publica. in Zool.*, 1927, xxxi, 79; PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 704; *Proc. Nat. Acad. Sci.*, 1927, xiii, 821.

while Irwin used Merck's medicinal and French's. The spectrophotometric readings of all these dyes showed them to be pure methylene blue. But since the spectrophotometric method is not refined enough to show the presence of 5% or less of trimethyl thionine in a solution of methylene blue, according to Holmes,[5] impurities of this amount in presumably pure solutions of methylene blue would escape detection. For this reason, the spectrophotometric analyses of all these dyes failed to show whether there were any impurities present.

However, in comparing the rate of penetration into *Valonia* of samples of Merck's and Clark's dye, it was found that Clark's penetrates much slower than Merck's under similar experimental conditions. Also the absorption maximum of sap into which Merck's dye had penetrated was 662mμ, as compared with 667mμ, the maximum obtained from Clark's dye in sap. This shows that the methylene blue penetrating from Clark's dye is more nearly pure than that penetrating from Merck's. Irwin[2] also notes that Clark's penetrates slower than Merck's and produces an absorption maximum at a longer wave length than Merck's or French's.[2]

Since trimethyl thionine penetrates faster than methylene blue and colors the sap more rapidly, the dye which penetrates the slowest was considered the purest. This was Clark's.

3. *Concentration of dye in external solution.* Irwin used from 3 to 25 times the concentration of dye used by the writer, or .01% and .04% (which is equivalent to .00031 M and .00125 M) as compared with .000047 M and .000095 M used by the writer. There is a greater amount of trimethyl thionine present in higher concentrations of methylene blue than in lower ones. Since the rate of penetration of trimethyl thionine is faster than that of methylene blue, experiments have been made which show how this error can affect the results.

4. *Light.* Irwin's experiments were done in an incubator "with air holes" while the writer's were done in normal conditions of illumination.

5. *Injury.* It is possible that the high pH values and concentrations used by Irwin may have caused injury to her plants. Her observation of loss of turgor is a point in case. Where no morphological changes can be observed as in the writer's experiments, the only way at present of determining whether a plant has been permanently injured is by noting how long it survives after having been replaced in its normal environment. Since all the plants used

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[5] **Holmes, W. C., *Stain Technology*, 1927, ii, 71.**

by the writer lived as long as the controls, no irreversible injury was manifest.

It has been shown that the apparent discrepancies in the findings of Irwin and those of the writer may be attributed to differences in methods. It seems best, therefore, in comparing results found by one investigator with those of another, to make sure that identical methods have been used, otherwise it is not justifiable to expect similar results. Differences creep in which are responsible for much confusion.

4263

Tissue Lactates and Blood Lactates as Affected by Muscular Exercise.

E. G. MARTIN, J. FIELD II AND V. E. HALL.

From the Laboratory of Physiology, Stanford University.

In dogs anesthetized with amytal the following preparations were made: 3 fore limb muscles and 2 hind limb muscles were prepared for rapid excision, with care to avoid injury to said muscles or interference with their blood and nerve supply. A carotid artery was cannulized, and a brachial vein and the inferior vena cava below junction with the renal veins were exposed. The blood vessels leading to both kidneys were tied off. Stimulating electrodes were imbedded under the skin of the back at the levels of the first lumbar and last sacral vertebrae.[1]

Continuous records of arterial blood pressure and of oxygen consumption were made. At the end of a 15-minute basal period a sample of arterial blood was drawn and one fore limb muscle rapidly excised and thrown at once into liquid air. Artificial exercise (confined to the hind quarters by the location of the electrodes) was then induced for 15 minutes. At the end of the exercise period blood samples were drawn from the cannulized artery, from the inferior vena cava (representing the outflow from worked muscles) and from the brachial vein (representing the outflow from non-worked muscles). One hind limb muscle (worked) and one fore limb muscle (non-worked) were excised rapidly and thrown into liquid air.

At the end of a recovery period approximately one hour in length blood samples were again drawn, as above, and one previously worked and one non-worked muscle excised and thrown into liquid air. Blood and muscle lactates were determined by the method of Friedmann, Cotonio and Shaffer.[2]

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[1] Martin, E. G., Field, J., and Hall, V. E., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 273.

The results of 5 experiments are summarized in Table I.

TABLE I.
a. Blood lactates; mgm. per 100 gm. blood.

Expt. No.	Before Exercise	Immediately after exercise			About 1 hr. after exercise		
	Artery	Artery	Vena Cava Inf.	Brachial Vein	Artery	Vena Cava Inf.	Brachial Vein
H 3	20.1	36.3	53.3	25.3	20.0	21.9	21.9
4	18.5	42.0	51.3	34.6	15.6	19.5	16.6
5	13.7	29.6	57.2	33.0	17.0	16.0	16.3
6*	21.5	32.0	38.0	26.7	24.8	24.5	26.6
8*	22.0	76.7	100.2	57.4	51.8	67.9	53.1

b. Muscle lactates; mgm. per 100 gm. tissue.

	Resting muscle	Worked muscle	Non-worked muscle	Worked muscle	Non-worked muscle
H 3	38.9	117.6	52.8	48.7	48.4
4	42.8	156.8	70.8	56.0	35.6
5	29.7	85.4	42.3	33.1	30.2
6*	41.2	75.7	61.1	78.2	40.2
8*	37.2	140.3	40.5	104.6	40.2

* Arterial pressure low during post-exercise period.

These experiments appear to us to show that lactates accumulate, sometimes to high concentration, in worked muscles during periods of exercise; that they tend to diffuse into the blood and be taken up by non-worked muscles during the same periods; that after exercise both worked and non-worked muscles gradually dispose of their lactate accumulations; that the partition coefficient for lactates between blood and tissue is not the same in dogs as assumed by some authors for man;[3] finally that arterial pressure is a significant factor in the disposition of the lactates formed during exercise.

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[2] Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

[3] Hill, A. V., Long, C. N. H., and Lupton, H., *Proc. Roy. Soc. B.*, 1924, xcvi, 438.

Illinois Branch.

Billings Hospital, December 18, 1928.

4264

Effect of Stimulation on Phagocytic Activity of Vascular Endothelium.

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From the Department of Pathology, Loyola University School of Medicine.

Opinion differs in regard to the ability of vascular endothelium to ingest particulate matter by phagocytosis. Maximow[1] regarded the phagocytic sinusoidal lining of such organs as the liver and spleen as a distinctive cell type consisting of flattened histiocytes quite different from the usual endothelium. Foot[2] now even suggests that the Kupffer cells of the liver may be monocytes anchored there. Gardner and Smith[3] failed to demonstrate any phagocytic activity of pulmonary endothelium. Seemann[4] also investigating the lung endothelium doubts its ability to phagocytose carbon. Lang[5] found carbon in the endothelium of granulation tissue but in quantity "incomparably smaller" than in the spleen and liver. India ink, as this investigator points out, becomes a rather coarse carbon suspension within the circulation. Although he speaks of the carbon being engulfed by the endothelium he does not regard the entrance of the coarse carbon particles into the endothelial cells as a phagocytic process. Finally he found that the endothelium transfers its carbon to extravascular pericytes (histiocytes) which are not of endothelial origin.

To test the phagocytic property of common endothelium, granulation tissue was produced by injecting into the groins and axillas of rabbits an alcohol-acetone mixture saturated with Sudan III. During the following week the animals were given intraperitoneally three 5 cc. doses of 2% trypan blue. A few days after the last trypan blue injection 2 cc. of India ink (Higgins) was injected into the ear vein and again after one hour an additional 2 cc. were given. The half-grown rabbits used for the experiments were lightly etherized and the needle kept in the ear vein for at least 10 minutes during the slow ink injection. The results obtained were based on the microscopic examination of tissues from rabbits living for 3 hours after the second ink injection at which time they were killed and the inflamed Sudan III tissue removed and fixed in both formalin and Zenker's fluid. The trypan blue granules are removed by the latter.

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[1] Maximow, A., *Arch. Path. and Lab. Med.*, 1927, iv, 557.

[2] Foot, N. C., *Am. J. Path.*, 1927, iii, 413.

[3] Gardner, L. U., and Smith, D. T., *Am. J. Path.*, 1927, iii, 445.

[4] Seemann, G., *Beitr. z. path. Anat. w. z. all g. pathol.*, 1925, lxxiv, 345.

[5] Lang, F. J., *Arch. Path. and Lab. Med.*, 1926, i, 41.

Results. The author[6] has tested the phagocytic activity of the endothelium of granulation tissue of animals that did not receive trypan blue and found that it may remove suspended carbon from the blood stream, but under the conditions of the experiments the number of carbon-marked endothelial cells was not very great. I found, as did Lang,[5] that the amount of carbon in the capillaries of the extremities is "incomparably small" in comparison with the spleen, liver and bone-marrow. After vital staining with trypan blue, the phagocytic activity of the capillary endothelium of granulation tissue is much increased and frequently as many carbon particles appear in the cytoplasm of these cells as are present in the sinusoidal endothelium of spleen and liver. The amount of free carbon within the capillary lumina, however, is always greater in these 2 organs than in the granulation tissue. The carbon is located in all possible positions as regards nucleus and is frequently placed at the ends and external to the nucleus. In the endothelia stimulated by the trypan blue one finds the coarse carbon stuck to the surface of the large endothelial cells, partially engulfed by them and finally completely incorporated within the cells. These stimulated cells ingesting the carbon often have an abundant cytoplasm and are equal in size to Kupffer cells and to the free phagocytes of the granulation tissue itself. So far as may be determined by examination of fixed tissue this entrance of microscopic particles into the cytoplasm of the endothelium is a true phagocytosis. The amount of carbon is greatest where the endothelial cells are largest and is scant in amount in places where these cells are thin.

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[6] McJunkin, F. A., *Am. J. Path.*, 1928, iv, 587.

4265

Dual Calorogenic Activity of Suprarenal Gland.

ALFRED E. KOEHLER. (Introduced by A. B. Hastings.)

From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

Our earlier studies have shown the beneficial effects of an epinephrin-free suprarenal preparation in a group of cases characterized by asthenia and fatiguability, functional circulatory disturbances and a faulty energy metabolism. These results, however, at first, were frequently variable. Occasionally a patient who improved with a certain preparation was made decidedly worse with the succeeding supply.

One of the methods that we have used in the study of the potency, fractionation, and stabilization of the active principles of the suprarenal gland depends upon the calorogenic effect of the preparations when administered to animals by mouth. The preparations which improved our patients showed a definite elevation of the basal metabolic rate of dogs, while those which had a depressing influence caused a lowering of the metabolic rate of the animals. This method of differentiation enabled us to obtain 2 suprarenal preparations of opposite calorogenic activities.

Fig. 1 shows the metabolic response of a dog after a single dose of the preparation which had been administered orally at the point indicated by the arrow.

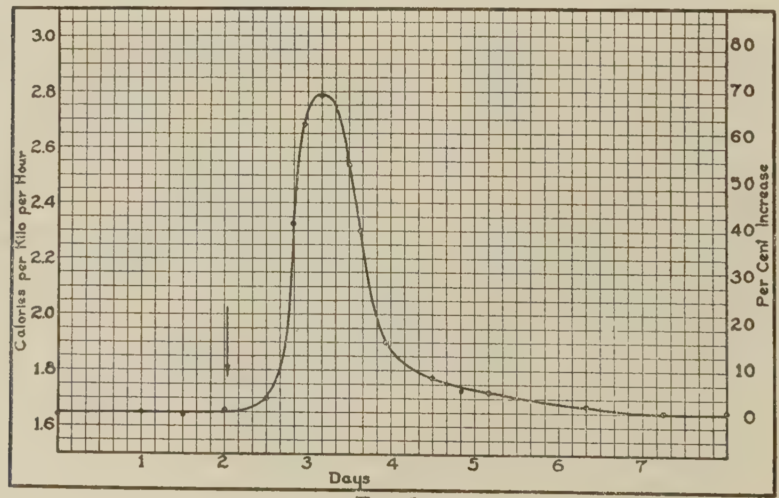

FIG. 1.

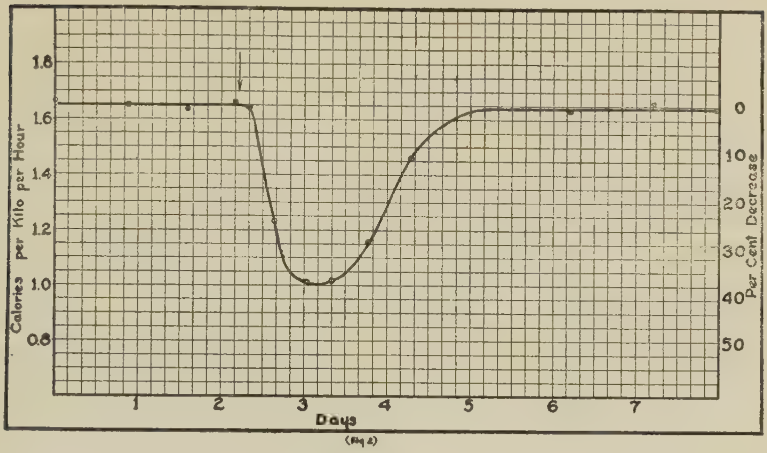

Fig. 2.

Fig. 2 shows the response of the same dog a week later to the depressing preparation. Although the metabolism was increased 70% in one case and depressed 38% in the other, the dog showed nothing unusual except a tendency to increased activity and restlessness in the one case and decreased activity and listlessness in the other.

The depressing preparation is now being tried in the treatment of hyperthyroidism with some encouragement.

We have, however, been particularly interested in the preparation which raises the metabolic rate and have attempted to devise other means of evaluating its efficacy in the treatment of the entity we have tentatively termed hypo-suprarenalism. In order to understand the mechanism underlying the improvement obtained in these cases and particularly to obtain accurate measurable criteria of improvement, we have studied the reaction of these patients to exercise both before and after treatment. For this purpose we have used a simplified type of work that can be carried out by the untrained individual during the metabolism test. While the patient is reclining he raises and lowers with the forearm a weight suspended over a pulley. The movement is synchronously timed with a metronome and the distance that the weight moves per minute is registered by a revolution counter connected to the pulley. Thus the patient does a type of accustomed work without suffering from fatigue.

Fig. 3 shows 2 such curves obtained on a patient suffering from hypo-suprarenalism. The continuous line shows the oxygen consumption before treatment while the broken line shows the metabolic

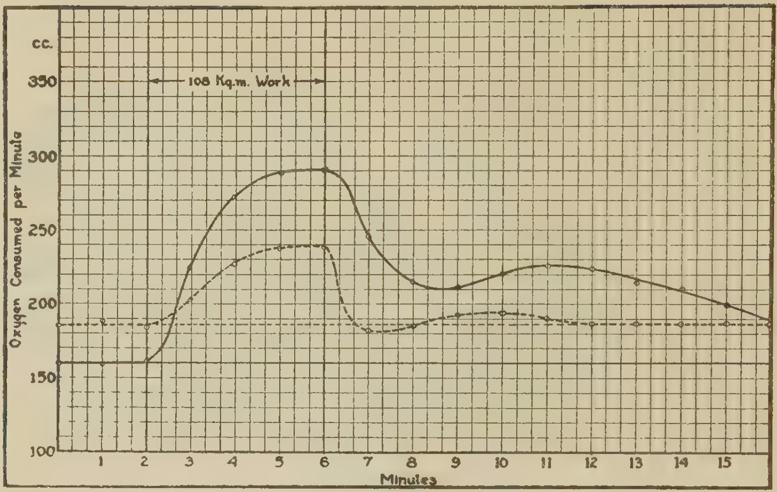

FIG. 3.

response 20 hours after a single dose of the preparation which raises the metabolic rate. The amount of work at each time was identical but the total amount of oxygen consumed varied greatly. The net mechanical efficiency increased from 3.6% to 14.8% after therapy. This gradually decreased again and nearly reached its former low level on the fourth day. In addition to giving the relative cost of work the work-metabolism test shows the large oxygen debt after cessation of work in this group of patients. The ratio of the oxygen used during work may be termed the D/W ratio. In the case illustrated in Fig. 3 this ratio before treatment was 1.8, but 20 hours after treatment it dropped to 0.8, about the normal ratio for this particular type of work.

The relatively large oxygen debt in these cases can at least in part be explained by the poor cardio-vascular response during this type of exercise. The pulse rate and pulse pressure was taken at frequent intervals during the tests and their product interpreted as the circulatory response. In the case illustrated the product before medication rose from 1200 to 1400, while the day after treatment it rose from 1400 before work to 2500 at the cessation of work.

4266

Virulence, Electrophoresis, and Conversion Characteristics of Bact. phaseoli sojense, S and R.

LEO RANE. (Introduced by I. S. Falk.)

From the Department of Hygiene and Bacteriology, University of Chicago.

The occurrence of "Rough" and "Smooth" strains among bacteria pathogenic for plants has been reported, and the conversion of R to S forms by passage through plants described.[1] The conditions associated with conversions of R to S and of S to R strains have been further investigated.

S to R Conversion: The culture of *Bact. phaseoli sojense* used in these experiments grows best at a temperature of 30° C. At this temperature, R and S strains are stable even after 5 months' incubation. When held at higher temperatures (up to 37.5° C.) S strains are transformed (not completely) to R. Growth in nutrient broth of hydrogen ion concentration between pH = 5.2 and 8.8 is without effect in changing the colony form. Cultivation in broth containing 1 to 20% homologous (S) anti-serum (rabbit) results in conversion of S to R (not completely); but in broth containing the heterologous (R) anti-serum, normal serum or no serum, no change is effected. If rapid serial transfers be made in broth containing 10% anti-S serum, the conversion of S to R forms is rapidly effected and is carried almost but not quite to completion. Similar conversion is effected by growth in peptone water containing from 2 to 15% peptone and is probably complete in the 10-15% solutions. Rapid transfer in nutrient broth or on agar slants or plates was without demonstrable effect.

R to S Conversion: Normal or rapid transfers in broth or on agar slants or plates did not effect conversion. Cultivation in the presence of homologous (R) anti-serum results in rapid but not complete R to S changes and serial transfer in the presence of 10% of this serum in the broth results in nearly complete transformation of the culture; whereas growth in the broth alone or in the broth containing normal or heterologous (S) anti-serum effects no change. A striking and nearly or entirely complete conversion of R to S is effected by growth on glucose agar. This conversion does not occur in glucose broth or other fluid glucose media (except when rapidly transferred), nor in solid or fluid media containing other sugars.

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[1] *Cf.* Sharp, C. G., *Bot. Gaz.*, 1927, lxxxiii, 113; also Link, G. K. K., Chap. xliv, and Falk, I. S., Chap. xlii, in "The Newer Knowledge of Bacteriology and Immunology," edited by E. O. Jordan and I. S. Falk, Chicago, 1928.

TABLE I.

Virulence for Manchu Soy Bean Plants Infections in Leaf Punctures.

Strain	% infection in 3 days	4 days	5-7 days	Average Size of Lesions
				mm.
R	31	49	61	1.37
R D	52	64	92	1.65
S	57	78	98	1.72

TABLE II.

Final serum dilutions	10	20	40	80	160	320	640	1280	2560	5120	Control
Agglutination of S, R, R D Types by S Immune Sera.											
S	++++	++++	++++	++++	++++	++++	++++	+++±	++	++	−
R D	++++	++++	++++	++++	++++	++++	++++	+++	+±	±	−
R	++++	++++	++++	++++	+++	+++	±	−	−	−	−
Agglutination of R, R D, S Types by R Immune Sera											
R	++++	++++	++++	++++	++++	++++	++++	+++	±	−	−
R D	++++	++++	++++	++++	+++	+±	±	−	−	−	−
S	++++	++++	++++	++++	++	±	−	−	−	−	−

Although stable, the R colonies differ in morphology when grown on solid media containing different sugars.

The R strain which has been converted to an S strain by growth on solid glucose media gives an unusually large colony and is subsequently stable and characteristically an S culture when carried on media containing or lacking glucose. The R to S transformation is effected in the presence of 0.3 to 20.0% glucose; but the colony is smaller in the presence of the highest concentrations of sugar. The effectiveness of the glucose is apparently not dependent upon impurities, as evidenced by experiments upon 5 commercial brands of the sugar of varying degrees of purity.

The R strains which have been converted to S by growth on glucose (dextrose)-containing medium, called the RD strains, show virulence, agglutination and electrical characteristics more like the typical S than the typical R strains. Tables I and II illustrate some experiments.

In agglutination tests with homologous and heterologous sera, results like those in Table II are obtained.

The electrophoretic charges or potential differences for 10 strains of each R, R D and S cultures are illustrated in Table III.

TABLE III.
Electrophoretic Potentials in μ/sec./volt/cm.[2]
(Migrations to the anode.)

Strain	
R	2.66
R D	4.04
S	4.21

In one experiment, S and R D cultures were found to be virulent for mice and the R cultures were not virulent. But it has been difficult to duplicate this experiment. Hence its significance is still uncertain.

4267

Experimental "Food-Poisoning" in Monkeys with Living Paratyphoid Bacilli.

G. M. DACK, E. O. JORDAN AND W. L. WOOD.

From the Department of Hygiene and Bacteriology, University of Chicago.

Previous experiments in this laboratory have shown: (1) that the symptoms of food-poisoning were not reproduced when enormous numbers of heat-killed paratyphoid bacilli were fed to monkeys and other animals;[1] (2) that similarly, no symptoms were produced when large amounts of heat-killed paratyphoid cultures were swallowed by human volunteers.[2] These results indicated that the thermostabile substance (or substances) long known to be toxic to animals on intraperitoneal inoculation, is not the substance (or substances) responsible for the gastro-intestinal outbreaks with which paratyphoid bacilli are commonly associated.

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[2] Using the "slide cell" method of Falk, I. S., Jensen, L. B., and Mills, J. H., *J. Bact.*, 1928, xv, 421.

Experiments have accordingly been undertaken using massive doses of living paratyphoid bacilli taken from agar cultures grown in Kolle flasks. These have been carried out with rhesus monkeys, and have yielded positive results. Monkeys of about 3½ to 4½ kilos, fed with from 95 to 816 billions of viable organisms, have shown symptoms of illness, such as sluggishness, loss of appetite and marked watery diarrhea. There was in all instances some loss of weight, but as a rule this was quickly regained. The diarrhea usually lasts 1 or 2 days, but in one instance persisted over 4 days. In no case did death occur, and the organisms fed were in no instance isolated from the blood, although daily attempts at blood cultures were made. One strain used (411) was of the Aertrycke type and was isolated in 1923 in a food-poisoning outbreak in New York City.[3] Large doses of heat-killed cultures of this organism had been given in previous experiments without causing any symptoms in man, monkeys or other animals.[1, 2] In the present series a saline suspension of organisms was prepared from several Kolle flasks, and was divided into 2 equal portions, one portion consisting of living organisms, the other portion being boiled for 20 minutes before being fed. Symptoms of "food-poisoning" appeared in animals fed with living cells, not in the others. Similar positive results were obtained with an Enteritidis strain, but monkeys fed with a living Proteus strain and with living *B. coli* strains showed no signs of illness. In 2 instances monkeys in which "food-poisoning" had been produced were fed again with the same strains 3 weeks later, and for the second time developed the typical symptoms.

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[1] Dack, Harmon and Jarra, *J. Prev. Med.*, 1928, ii, 461.

[2] Dack, Cary and Harmon, *J. Prev. Med.*, 1928, ii, 479.

[3] *Cf.* Salthe and Krumwiede, *Am. J. Hyg.*, 1924, iv, 23.

4268

Effect of Diversion of Bile into Vena Cava and Portal Vein in Dogs.

LESTER R. DRAGSTEDT AND BERT SPURRIER.

From the Department of Surgery of the University of Chicago.

It is definitely established that the dog may survive complete obstruction to the common bile duct for many months in comparatively good condition. On the other hand the injection or leakage of any considerable amount of bile into the peritoneal cavity causes prompt toxemia and death. The bile salts are apparently the principal toxic elements in the bile.[1] The present experiments were devised to determine the effect of diverting the biliary flow directly into the portal and systemic circulation. Dogs were used and all operations done under morphine ether anesthesia. In 3 experiments glass cannulae were placed in the common bile duct and left renal vein and connected by oiled rubber tubing. A nephrectomy was previously done. These 3 dogs died in 3, 5, and 7 days respectively. At autopsy a large amount of bile stained fluid was found in the peritoneal cavity and the rubber tube obstructed by a blood clot. Death was probably due to bile peritonitis. In 4 experiments the common bile duct was doubly ligated and divided, the gall bladder dissected free from its liver bed, and an anastomosis made between the fundus of the gall bladder and the vena cava, using a technique similar to that for the Eck-fistula. All 4 animals recovered from the anesthetic but remained weak and depressed. Two were unable to stand. All of these dogs died in 18, 22, 36, and 48 hours respectively. In 2 animals in which a preliminary cholecystectomy had been done 3 weeks previously and in consequence of which the common bile duct was greatly dilated, an anastomosis was made between the common bile duct and the portal vein in one and between the bile duct and vena cava in the other. Both of these dogs died in 20 hours. Since the authors have previously done over 50 Eck-fistula operations with less than 10% mortality it is not probable that death in these experiments is due to operative trauma, but rather to some toxic element in the bile, which is turned back into the blood. It seems probable that this substance (possibly bile salts) is not formed or does not escape from the liver in obstructive jaundice.

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[1] Horall, O. H., and Carlson, A. J., *Am. J. Physiol.*, 1928, lxxxv, 591.

4269

Effect of Liver Autolysis in Vivo.*

JAMES C. ELLIS AND LESTER R. DRAGSTEDT.

From the Department of Surgery of the University of Chicago.

It has been concluded by Mason, Davidson, *et al*,[1] that aseptic autolysis of the animal's own liver placed in the free peritoneal cavity, under aseptic conditions, causes death of the animal in 15 to 18 hours. This work has been repeated by Wangensteen and Waldron,[2] with essentially the same conclusions.

In our first series of experiments the blood supply to the small lobe of the liver to the left of the gallbladder was ligated, and the liver left *in situ*. Careful aseptic technique was practiced. All of these animals died in 12 to 20 hours. Blood chemical studies were made of the non-protein nitrogen, the urea nitrogen, and the whole blood chlorides. No significant changes were noted in the blood chemistry. At autopsy the ligated lobe of liver was always gray-brown friable, necrotic, and often contained gas. In only one instance was a culture of the liver made at post-mortem. A Gram positive, spore bearing, anaerobic organism was obtained.

In a second series of experiments the entire liver of an animal was removed under aseptic technique, and pieces carefully cultured. All cultures of fresh liver were positive for a Gram positive anaerobic bacterium, except in 2 instances. Such fresh liver was placed, using careful aseptic technique, either into the peritoneal cavity, or between the muscles of the abdominal wall of other animals. Death of the animals followed in 18 to 30 hours. At autopsy the liver was gray-brown, necrotic, friable, and gas-containing. Anaerobic bacteria were present in all cultures of the liver. Chemical blood studies revealed no significant change.

The autolytic enzymes of fresh liver were destroyed by heating to a temperature of 75°-80° C. for 15 to 20 minutes. A Gram positive anaerobic organism was cultured from the liver after heating. Placing such liver into the abdominal wall or into the peritoneal cavity caused death of the animals in 24 to 36 hours. The anaerobic organism was identified at post-mortem. The liver at post-mortem was firm, gray-brown and smooth.

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Mason, Davidson, Mathews, and Rastello, *J. Lab. and Clin. Med.*, 1924, x, 622.

[2] Wangensteen, O. H., and Waldron, G. W., *Arch. Surg.*, 1928, xvii, 430.

Liver that had been autoclaved at a pressure of 15 pounds for 15 minutes was sterile. Pieces of such liver were placed into the peritoneal cavity of other animals without causing death. Some of such autoclaved liver was reinfected by organisms isolated in pure culture from fresh liver. Such a piece free in the peritoneal cavity or abdominal wall caused the death of the animals within 24 hours.

Foetal liver, secured aseptically from ceserean section pups was proven sterile by culture. When placed into the peritoneal cavity of other animals it was autolyzed completely without injury to the animals.

Conclusions. (1) The uncontaminated liver of normal healthy adult dogs regularly contains anaerobic bacteria. (2) It is probable that the experimental so-called "*In Vivo* aseptic autolysis of the liver," is always complicated by this infection, and that this is the cause of death. (3) *In vivo,* aseptic autolysis of fetal liver (proved sterile by culture) does not produce toxic effect. (4) Autolytic enzymes of liver may be destroyed by heating, but such pieces of liver cause the death of an animal in 24 to 36 hours. An anaerobic, spore-bearing bacterium may be recovered from the liver at autopsy. (5) Autoclaved liver is sterile, and causes no harm to an animal if placed into the peritoneal cavity. If autoclaved liver is re-infected with the anaerobic organisms before being placed into the peritoneal cavity, or abdominal wall, death results within 24 hours.

4270

Fatal Effect of Total Loss of Gastric Juice.*

LESTER R. DRAGSTEDT AND JAMES C. ELLIS.

From the Department of Surgery of the University of Chicago.

The present experiments were undertaken to determine the effect of the total loss of gastric juice from the body under conditions not complicated by obstruction such as are present after closure of the pylorus. Recourse was made to a method described by Lim, Ivy, and McCarthy[1] with this important modification that the vagus nerves to the isolated stomach were left intact. The stomach was cut across at the cardia and the pylorus. The cardiac end of the stomach was then inverted and closed and the open pyloric end brought to the surface as a fistula. An anastomosis was made between the lower end of the esophagus and the duodenum. Great care was taken in sectioning the cardia not to interfere with the vagus fibers to the stomach and as much of the blood supply to the isolated stomach was left as was technically possible. To date we have made 3 such preparations but the data to be presented here are from a single animal. At autopsy the vagus nerves to the isolated stomach in this animal were found intact. A daily record was kept of the water and food intake (food exclusively milk and muscle), the urinary output, and the quantity and composition of the gastric secretion collected from the isolated stomach. In addition blood was drawn at the end of each 24 hr. period and determinations made of the CO_2 combining power of the blood plasma, the blood pH, and the NPN, urea N, and chloride concentration.

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Lim, Ivy, and McCarthy, *Quar. J. Exp. Physiol.*, 1925, xv, 13.

The following points have been established: (1) The total loss of the gastric secretion from the body causes death in the dog with symptoms of weakness, anorexia, oliguria and profound depression. (2) Accompanying these symptoms and proportionate to their severity occurs a prompt fall in blood chloride, an increase in the CO_2 combining power of the plasma, an increase in the pH, and a late increase in the NPN and urea N. (3) The symptoms may be relieved and the blood chemistry returned toward the normal by the intravenous injection of from 2,000 to 3,000 cc. of Ringer's solution or 0.9% NaCl solution per 24 hrs. (4) The isolated stomach, with intact blood and vagal supply, will frequently secrete in 24 hrs. from 1000 to 1600 cc. of gastric juice with a chloride concentration of 0.5 to 0.55%. There is thus excreted by the gastric mucosa in 24 hours more than twice the calculated amount of chloride in the circulating blood. It is this loss of chloride which is chiefly responsible for the death of the animal. (5) The gastric mucosa can continue to secrete gastric juice of high free and total acidity (0.312% and 0.363% respectively) even when the blood chloride has been reduced to less than a third of its normal concentration (108 mg. of Cl per 100 cc.) and an extreme alkalosis exists (Plasma CO_2 104). Under these conditions, however, the volume of secretion is lessened. (300 cc. per 24 hrs.). It should be mentioned that this is continuous secretion since the animal is depressed and refuses all food. The ordinary stimuli for secretion are thus absent. (6) When as a result of alkalosis, hypo-chloremia, and absence of food taking the volume of juice is depressed, the intravenous injection of Ringer's solution causes a pronounced increase in secretion (from 300 cc. per 24 hrs. to 900 cc. and this without the stimulus of food).

(7) When the animal is in severe hypo-chloremia and considerably dehydrated, the secretion of urine being greatly lessened, it apparently does not experience thirst for it drinks but little water. If then it is given a 1000 cc. of Ringer's solution intravenously, it promptly becomes thirsty and drinks large quantities of water. (8) The body temperature remains normal throughout the periods of dehydration. (9) Evidence of tetany has been uniformly absent except that occasional slight twitchings of the legs have been observed during the intravenous injection of Ringer's solution. For the most part the deviation from the normal has been in the direction of depression rather than excitation.

4271

Action of Iodized Oils on Serous Membranes.

L. A. CRANDALL AND E. L. WALSH. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Iodized oils (Lipiodol "Lafay", and Lipoiodin "Ciba") have been injected into the pericardium, pleura, peritoneum, and joint cavities in an endeavor to study the pathogenicity of these substances. Dogs were used throughout. Ten animals were injected intrapericardially with 15 to 20 cc. iodized oil; in each case the dog developed a sterile pericarditis with marked effusion. Death was in most cases due to a pneumonia which was probably secondary to an embarrassed circulation caused by the pericardial effusion. The pericardium was covered with a fibrinous exudate resembling that of the "shaggy heart" of pneumonia; there was thickening of the pericardium and it appeared congested. A few adhesions were commonly present, especially about the auricles. The exudate consisted of from 90 to 300 cc. of creamy fluid, which on microscopic examination showed oil droplets, fibrin, and leucocytes (chiefly lymphocytes). Direct smears of the effusion were always negative for bacteria, as were cultures. Microscopic examination of frozen sections stained with Sudan III and of ordinary hematoxylin and eosin preparations showed the fibrin layer covering the inner surface of the pericardium to be loaded with leucocytes and oil droplets. The pericardium itself contained many leucocytes and a few red blood cells. The first few layers of muscle fibers under the pericardium had undergone fatty degeneration.

Two dogs injected with olive oil as controls were negative when killed after 28 and 30 days, respectively. Two poppy-seed oil controls showed the same picture as that found in the animals injected with iodized oil

Seven animals were injected with from 10 to 18 cc. intrapleurally. In general, the results of intrapleural injections resemble those of intrapericardial except that the irritative action is not as marked. Some of the animals died in 10 days to 2 weeks; others survived 3 weeks and were then killed. Those least affected show only a very mild pleuritis with a few adhesions and no effusions; others show marked congestion, many adhesions, and 40 to 100 cc. of effusion. Direct smears and cultures were negative in all cases. Microscopic examination is essentially the same as in the intrapericardially injected animals.

Four dogs were injected intraperitoneally, using 18 cc. of iodized oils for each animal. One died of intercurrent pneumonia in 3 days; the oil was still in the abdomen and no pathology was present. The remaining 3 were killed in from 12 to 22 days; in no case was any inflammatory reaction found, and direct smears showed only oil with an occasional cell.

The right knee and shoulder joints were injected in each of 3 dogs, each joint receiving 2 cc. No difficulty in using the joints was exhibited by any of these animals. When killed in from 22 to 26 days a very mild reaction was found. The joint cartilages showed no change, but there were areas of fibrin deposit on the capsule which microscopically showed leucocytic infiltration. All joints were sterile.

The iodized oils call forth the greatest reaction from the pericardium, less from the pleura, very little from the joints, and none from the peritoneum. This suggests that the amount of motion of a tissue is a factor in its susceptibility. Certainly the marked irritating action of these substances on the pericardium proves that iodized oils are not harmless in all parts of the body.

We suggest the intrapericardial injection of iodized and plain oils as an excellent test of their pathogenicity. Further studies will be performed along this line.

4272

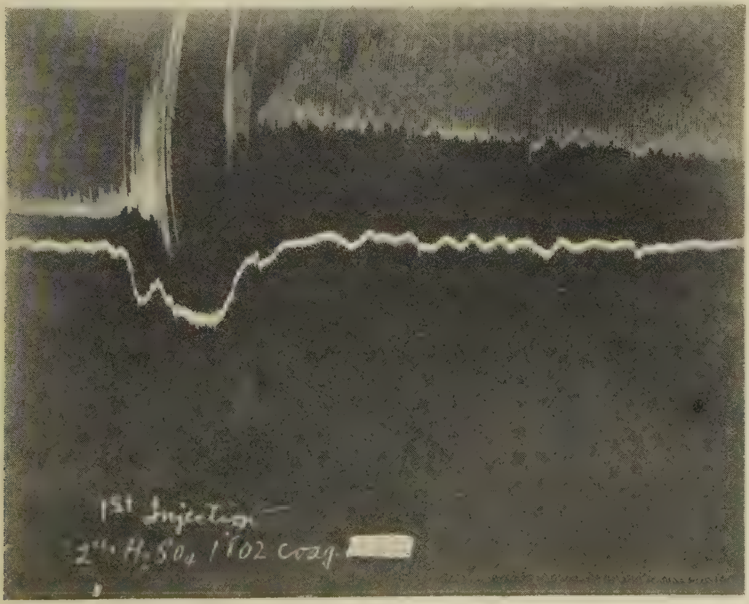
A Vaso-Depressor Phenomenon Caused by Some Cholecystokinin Preparations.

A. C. IVY, H. C. LUETH AND GRANT KLOSTER.

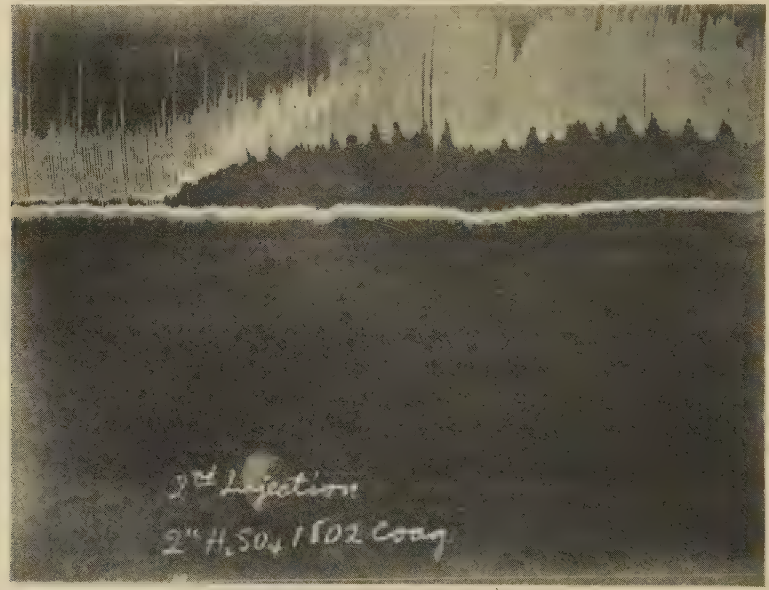
From the Department of Physiology and Pharmacology, Northwestern University Medical School.

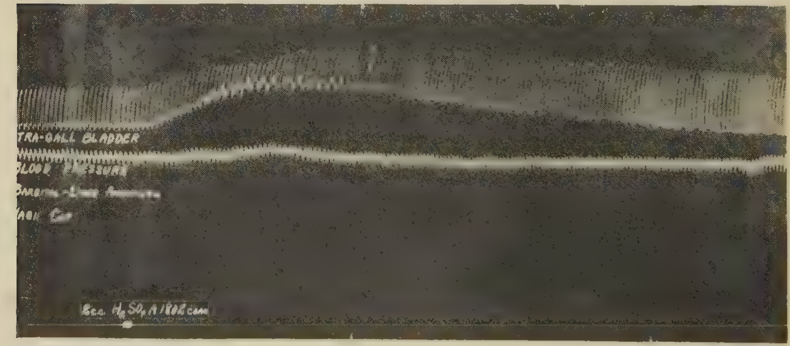
Throughout our work on the purification of cholecystokinin, we have not infrequently observed that certain preparations on their first injection into some dogs under barbital anesthesia cause marked contraction of the gall-bladder, followed by a slow fall in blood pressure (30-60 mm. Hg.) with a gradual return to normal, and a stimulation of respiration (which does not always occur) that appears soon after the blood pressure begins to fall. (The contraction of the gall-bladder persists from 10 to 20 minutes after the blood pressure and respiration have returned to normal. A second injection of the same material some 10 or 20 minutes later causes the gall-bladder to contract, but has no effect on the blood pressure and respiration. Apparently some rapid compensatory change or readjustment has occurred in the animal.

Two possible explanations of this phenomenon have occurred to us: one, that a rapid and violent contraction of the gall-bladder stimulates vagus endings and leads to the change in blood pressure and



respiration; the other, that there is some toxic element in the preparation to which the dog rapidly immunizes or adjusts itself. This latter possibility seems reasonable since our records show that we have only observed this phenomenon when the cholecystokinin was prepared from hog's intestines, and it has not occurred when dog's intestines were used. The dog's intestines used are very fresh, but we have to wait some 15 or 20 minutes after the death of the hog before we can get the intestine. Our hog material has never been as pure as our dog material.





Tracing 1 shows the response to the first injection of our preparation. In some dogs the fall in pressure occurs later and more slowly and the rise more slowly. Tracing 2 shows the response to the second injection approximately 20 minutes later in the same dog. Tracing 3 shows the response in a dog with the vagi cut.

The first hypothesis was tested as follows: we used a preparation that was known to produce the above phenomenon in some dogs. We made injections under barbital anesthesia in 20 dogs with vagi cut and in 10 without the vagi cut. The phenomenon occurred in 5 of the 10 dogs with the vagi intact. In the 20 dogs with the vagi cut, the phenomenon occurred in 4 cases. In one, the fall of blood pressure amounted to only 20 mm. Hg., but was a typical slow fall and return to normal. In another the fall was marked, 80 mm. Hg., and in the other the fall was 50 mm. Hg. If we had not used 20 dogs, we would not have observed the last 3 cases, and would probably have been led to an erroneous conclusion. These results show that this phenomenon will occur after the vagi have been cut. We have also observed a fall occur in one dog after atropine.

Our results indicate that this phenomenon is due to some toxic substance against which the body rapidly immunizes or adjusts itself.

Abel and Geiling[1] report that some 6% of dogs have a natural immunity to "peptone" and that dogs that survive "peptone" shock are immune to repeated injections. The causative agent of the phenomenon we have observed is precipitated by NaCl from acid solution and by 5% trichloracetic acid, and is not coagulated nor denatured by heating at boiling temperature for 30 minutes.

4273

Effect of Histamine on Gall-Bladder Evacuation.

H. C. LUETH, B. H. ORNDOFF AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

It has been shown in a previous article that 0.4% HCl in the duodenum causes the gall-bladder to contract on the basis of a humoral mechanism.[1] It has also been shown that histamine when injected intravenously causes the gall-bladder to contract, but the contraction is a mirror-image of the fall in blood pressure which led us to believe that the contraction was due to the fall in blood pressure.

It occurred to us that since it is well known that histamine subcutaneously causes gastric juice to be formed, such an injection might lead to gall bladder evacuation by the passage of the gastric juice into the duodenum. The gall-bladder of 5 normal men, 5 patients, and 10 dogs was visualized by the phenoltetraiodophthalein technique. One milligram of histamine was then injected subcutaneously and pictures of the gall-bladder were made at 30 minute intervals for one and a half hours. It was found that in the normal men the shadow was not changed in any way. In 2 of the dogs there was a slight decrease in the shadow, in the others it was not changed. In 3 of the 5 patients there was no change in the gall-bladder; in 2 there was a slight, but insignificant change; but in one of the patients, the gall-bladder emptied so completely within 30 minutes that only a narrow bandlike shadow remained.

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[1] Abel and Geiling, *J. Phar. Exp. Therap.*, 1924, xxiii, 1.

[1] Ivy, A. C., and Oldberg, Eric, *Am. J. Physiol.*, 1928, lxxxvi, 599.

Our observation shows that the subcutaneous injection of one milligram of histamine only occasionally leads to evacuation of the gall-bladder, and hence it offers no possibility of being a gall-bladder function test. The question logically rises that since on theoretical grounds histamine would serve to evacuate the gall-bladder, why does it not act uniformly? Several possibilities might be suggested: first, the gastric juice formed is not ejected into the duodenum in sufficient quantities to act, which is likely since it was found[1] that more than 10 cc. of 0.4% acid had to be introduced into the duodenum of the barbitalized dog to cause gall-bladder contraction. Second, the gastric juice may have been neutralized by regurgitated intestinal contents. Third, the histamine may cause an increase in tone of the sphincter of Oddi or the duodenal musculature which would prevent the evacuation of bile from the gall-bladder. Other suggestions might be presented, but we think these 3 possibilities are the most likely.

4274

Emptying of Gall Bladder in Children.

E. A. BOYDEN AND C. L. BIRCH.

From the Departments of Anatomy and Medicine, University of Illinois, Chicago.*

While studying catarrhal jaundice in children, it was found necessary to ascertain the emptying time of the gall bladder in non-jaundiced individuals of comparable age. This paper deals with 3 normal cases. In each instance 75 mg. of "Iodeikon" per kilo were administered orally, in accordance with the recommendation of Dr. Evarts Graham. Children over 5 years old were given the usual capsules, but in babies the dye had to be administered in the form of an emulsion (specially prepared by Swan Myers Co. of Indianapolis). Up to the present time we have been unable to secure intestinal absorption of the emulsion in babies, but visualization of the gall bladder in children of 5 to 9 years has been readily obtained. The first few cases (Fig. 1) seem to indicate that the rate of emptying does not differ greatly from that of adults.[1] This seems reasonable in view of the fact that from infancy on, milk forms a principal constituent of the diet.

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* The patients were secured from the Department of Pediatrics through the cooperation of Dr. Julius Hess and his assistants.

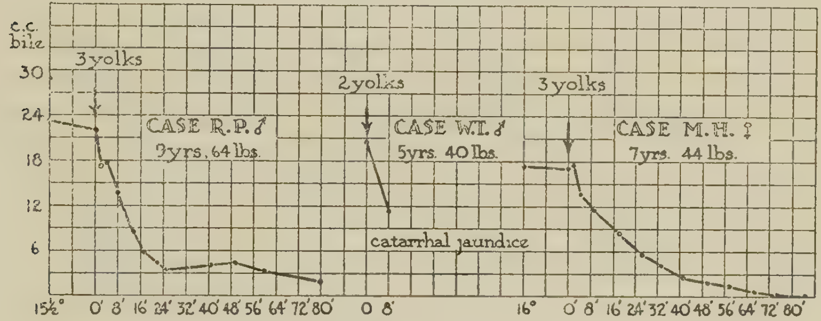

Fig. 1.

Graphs showing emptying of the gall bladder after a test meal of egg-yolk. In case *W. T.*, a patient recovering from icterus, the X-rays were made one day after the stools became stained with bile.

In connection with these findings it is interesting to note that Halpert[2] has recently called attention to the greater competency of the Heisterian valves in children. But if the cases shown in Fig. 1 are representative, the valves would not seem to offer appreciable obstruction to the flow of bile. Another feature of interest is the relatively large size of the gall bladders shown in Fig. 1, in which the average volume of body and fundus (20 cc.) exceeds that of the 8 smallest adult gall bladders (18 cc.) recorded in Tables I and II (l. c.).[1]

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[1] Boyden, E. A., *Anat. Rec.*, 1928, xl, 147.

[2] Halpert, Bela, *Arch. Path.*, 1928, vi, 623.

4275

Tonus Changes in the Gall Bladder Induced by Faradic Stimulation.

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In previous publications it has been shown that a stimulus that usually causes contraction of the human gall bladder may sometimes induce the reverse effect.[1, 2] This has led us to believe that evacuation of the gall bladder is regulated in part by a reflex mechanism, and that from the standpoint of biliary stasis the reflex control may be more important than any other. Accordingly we have undertaken to ascertain whether reflex inhibition of the gall bladder musculature can be induced by stimulating the intramural portion of the common bile duct with a weak faradic current. Preliminary observations show that if such a current is applied when the gall bladder is under contraction (either spontaneous or after ingestion of egg yolk) tonus diminishes and the column of iodized oil in the gall bladder drops. This work is being supplemented by further experiments dealing with other sensory areas and degrees of stimulation, and with the gall bladder in different initial states of tonus.

4276

Nature of Local Immunity in Lungs of Rabbits Immunized Against Pneumococci.*

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From the Department of Hygiene and Bacteriology and the Department of Pathology, University of Chicago.

In a series of experiments designed to study the pathogenesis of experimental pneumococcus pneumonia in rabbits by the method employed by Lamar and Meltzer,[1] Winternitz and Hirschfelder[2] and

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[1] Boyden, E. A., and Parmacek, L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 462.

[2] Boyden, E. A., and Saunders, A. M., *Ibid.*, 1928, xxv, 458.

* This is a preliminary report on one of a series of studies on respiratory diseases carried out in connection with the Influenza Commission, established and financially aided by the Metropolitan Life Insurance Company of New York.

[1] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

[2] Winternitz, M. D., and Hirschfelder, H. D., *J. Exp. Med.*, 1913, xvii, 657.

Armstrong and Gaskell,[3] it was found that the intrabronchial insufflation of a broth culture of virulent type I pneumococcus leads to infection of the lungs, with a marked rise in body temperature within 24 hours, usually followed by death of the animal in 1 to 3 days. While pneumococci were recovered from the peripheral blood in only a few of the rabbits before death, blood agar plate cultures of the individual lobes of the lungs, the heart's blood and trachea, taken at autopsy, revealed large numbers of pneumococci. The leukocyte counts were not particularly significant, in that marked increases in numbers were observed in some rabbits and decidedly fewer numbers in others of the same series.

The pathological study of the lung tissues from these animals presents a fairly uniform picture. There is a marked acute inflammatory reaction, the polymorphonuclear leukocyte being the predominant cell of inflammation, with macrophages and lymphocytes in lesser numbers. Eosinophiles are only rarely seen. A diffuse edema of the lungs is common; when areas of consolidation occur they tend to be of a lobular type or the result of dense infiltrations of polymorphonuclear leukocytes in the perivascular and peribronchial lymphatics. The pulmonary lymph follicles are not unusually hyperplastic.

In another series of experiments in which an attempt was made to balance the resistance of the rabbits against the virulence of the pneumococci by partial immunization as reported by Wadsworth,[4] the course of events was found to be entirely different.

When rabbits were injected daily from 1 to 10 days in the submucosa of the cheek with 1 minim amounts of a heat killed saline suspension of type I pneumococci,[5] or the nose and mouth sprayed daily with the same vaccine, and this was followed 10 to 20 days later by an intrabronchial insufflation of virulent type I pneumococci, a definite immunity sufficient to preserve the life of the rabbit was observed to be present in those animals which had received 5 or more vaccinations. Furthermore, the body temperature and leukocyte reactions were not accelerated to any marked degree and blood cultures were sterile. In a series of vaccinated rabbits which were killed at 2 and 3 day intervals following a test dose of virulent pneumococci by intrabronchial insufflation, blood cultures of the lungs, heart's blood and trachea were uniformly negative, in contrast to the great numbers of pneumococci present in the unvaccinated controls.

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[3] Armstrong, R., and Gaskell, J. F., *J. Path. and Bact.*, 1914, xix, 126.

[4] Wadsworth, A., *Am. J. Med. Sci.*, 1904, cxxvii, 851.

[5] Stuppy, G. W., and Falk, I. S., *J. Prev. Med.*, ii, 175.

The histological picture of the inflammatory reaction in the lungs of the vaccinated rabbits is the following: the predominant cell of inflammation is the histiocyte, which is present in large numbers. Many of these contain mitotic figures and hyperchromatic nuclei, and the alveolar spaces are filled with such cells, together with precipitated protein from edema. In many areas, both within the lumina of blood vessels and around them, or in collections in the tissues, are numerous eosinophiles with polymorphous nuclei. Polymorphonuclear leukocytes are distinctly in the minority as compared with histiocytes. The pulmonary lymph follicles in many instances, and particularly near the hilus, are markedly hyperplastic with prominent germinal centers rich in mitotic figures.

It is evident then, that the previous treatment of these rabbits with a killed saline suspension of pneumococci has been followed by an altered reactivity upon later intrabronchial insufflation of living virulent pneumococci of the same type. This altered reactivity is distinctly an increased immunity, inasmuch as the animals show less marked reactions and usually recover from the intrabronchial insufflation. It is interesting to recall that Bull and McKee[6] have reported the development of sensitization to pneumococcus autolysate following pneumococcus infection after intranasal inoculation.

The rapid cellular response of histiocytes and eosinophiles indicates that the reaction is allergic in nature,[7] and is evidence of a definite, increased local immunity of the pulmonary tissues. This appears to be somewhat analogous to the findings of Gay[8] *et al.* in regard to the local immunity obtained in experimental streptococcus infections, especially so far as the response of the histiocyte or clasmatocyte is concerned. It would appear that the previous introduction of pneumococcus antigen had so sensitized the pulmonary tissues that their reactivity was increased to the extent that the later insufflation of the living organisms was followed by a rapid and vigorous exudative and proliferative reaction, with the histiocyte as the principal cell of defense.

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[6] Bull, C. G., and McKee, C. M., *J. Am. Med. Assn.*, 1928, xci, 396.

[7] Maximow, A. A., *Arch. Path. and Lab. Med.*, 1927, iv, 588.

[8] Gay, F. P., *Arch. Path. and Lab. Med.*, 1926, i, 857.

4277

Circuit Transmission and Interference of Activation Waves in Passive Iron Wire.

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The pure commercial soft iron wire known as Armco,* when rendered passive in nitric acid of 70-80 v. %† transmits activity in the usual manner whenever the wire is activated locally by any means, such as scraping with glass or touching with zinc. The resulting activity in such strong acid is temporary, each area of the wire becoming active in its turn as the state of activity spreads, and then reverting to the passive state. Both the transmission and the repassivation are electrochemical processes; any area on becoming active forms the anode of a local electric couple, the adjoining passive area forming the cathode; the latter is then automatically activated through the destruction of the passivating surface film by electrochemical reduction; and by repetition of this process the state of activity spreads rapidly over the whole wire. The reversal or repassivation is a result of the reformation of the surface film by oxidation of the exposed metal; in strong acid (above 60 v.%) the reaction of the metal with the acid is self-limiting, by reason of the electrochemical oxidation which follows automatically in any area of metal as it becomes active and therefore anodal. This oxidation reforms the film and passivates the metal. Accordingly, in strong acid the reaction at any point of the wire lasts for only a brief time, which is the briefer the stronger the acid, *e. g.*, a small fraction of a second in fully recovered Armco wires in 80 v.% HNO_3 at 20°.

When a passive Armco wire in strong acid is touched with zinc at any point a restricted area or "wave" of activity, indicated by a darkening of the metallic surface and a slight effervescence, is seen to pass along the wire in both directions from the contact. The velocity and length of this wave vary with the concentration of acid, the temperature and the interval since the previous activation. In HNO_3 of 78 to 80 v.% at 15°, under the conditions described below, the wave retains an approximately constant length of 12 to 15 cm. and travels at *ca.* 15 cm. per second. During the time (*ca.* 1 second) occupied by the reaction at each point the surface film is successively broken down by cathodic reduction and reformed by anodic oxidation. Alternate reduction and oxidation thus constitute the chemical cycle at each region of the surface as the activation wave passes.

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* American Rolling Mill Co. The carbon content is given as less than 0.1%.

† Volumes of acid (C. P. HNO_3 of sp. gr. 1.42) in 100 volumes of solution.

Immediately after repassivation iron wires of all kinds are resistant to activation and transmit imperfectly and slowly. This non-reactive phase (comparable with the refractory phase of living tissues) passes off by degrees, within a time which varies, according to the kind of wire, from some minutes (steel wire)[1] to a second or less (pure iron wire).[2] Armco wire is remarkable in having a very brief period of recovery, of less than one second in HNO_3 of 70-80 v.% at 20°; after this interval it again transmits activation waves to an indefinite distance as before.

This combination of rapid repassivation and prompt return of transmissivity shown by Armco wire in strong acid makes it possible to obtain in rings of wire activation waves of limited length which travel at a moderate rate; by the time such a wave has completed the circuit of the ring the wire has recovered its transmissivity and the wave continues its circular motion; under favorable conditions this circuit transmission lasts indefinitely.

In order to demonstrate this phenomenon a circular loop of wire 22 cm. in diameter is suspended by thin glass hooks in a ring-shaped volume of acid 2 cm. wide and 3 cm. deep, contained in a circular trough made by cementing a section of glass cylinder *ca.* 5 cm. high and 20 cm. outside diameter to the bottom of a crystallization dish of 24 cm. diameter. A small piece of platinum wire is inserted where the 2 ends of the wire are hooked together to form the loop; this obviates irregular action at the junction. The temperature is regulated by a water bath. A suitable concentration of acid is 75 to 80 v.%.

Interference of activation waves is shown when the passive wire is touched with zinc at any point. The 2 diverging waves meet at the opposite side of the ring and there instantly and completely extinguish each other, the whole wire becoming again passive. The experiment can then immediately be repeated with the same result. Interference can also be readily demonstrated in a long straight wire immersed in a bath of acid and touched simultaneously at opposite ends, or in a vertically suspended wire over which a thin stream of acid is kept flowing from a siphon. The effect is a result of the intersection of the oppositely oriented local circuits of the 2 activation waves; the associated electric currents annul one another by compensation and the associated chemical action ceases. A similar interference of excitation waves in living tissues has long been known, and is shown clearly in rings cut from large hearts[3] or medusæ.[4]

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[1] Lillie, R. S., *J. Gen. Physiol.*, 1920, iii, 107; 1925, vii, 473.

[2] Lillie, R. S., *Science*, 1928, lxvii, 593.

Circus transmission in such rings of tissue is shown when a single excitation wave is started in one direction; in Cassiopea such "trapped" waves may last for days.[5] In the iron wire single activation waves are readily obtained by blocking the transmission of one wave with platinum-tipped forceps near the contact with zinc; the other wave continues its movement and when the platinum is withdrawn is left isolated in the wire. Its motion then continues uniformly round and round the wire until the materials are depleted or some other interfering condition arises. At any time its progress can be stopped by contact with platinum.

The following are typical speeds of transmission observed in a single wire in the same bath of acid (79 v.% HNO_3) at different temperatures:

Temperature	*Speed (cm. per sec.)*
8°	8
13-14°	13-14
18°	24-25
23°	33-35

The Q_{10} values (2.5 to 3) are higher than those usually observed with nerve or fully recovered passive iron. This is because at the time of the return of the wave on its circuit the wire is still incompletely recovered. The speed of transmission is slower during the relative refractory period than later (as is also the case in heart muscle[6] and nerve[7]); at any time the speed is limited by the degree of recovery attained. These observations indicate in reality the temperature coefficient of recovery, which is high in both living tissues and passive iron;[8] the coefficient of transmission in fully recovered wires is much lower ($Q_{10} = 1.4\text{-}1.5$) just as it is in nerve and muscle.

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[3] Mines, G. R., *J. Physiol.*, 1913, xlvi, 349.

[4] Mayer, A. G., *Publ.* 102. *Carnegie Institution*, 1908, 113.

[5] Mayer, A. G., *loc. cit.;* Harvey, E. N., *Carnegie Yearbook*, 1911, x, 130.

[6] Mines, G. R., *loc. cit.*

[7] Forbes, A., Ray, L. H., and Griffith, F. R., Jr., *Am. J. Physiol.*, 1923, lxvi, 553.

[8] Lillie, R. S., *loc. cit.*, 1925.

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4278

The Demethoxylation of Lignin in the Animal Body.

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We recently reported[1] that lignin when fed to a dog or cow causes a substantial increase in the quantity of benzoic acid eliminated in the urine. Since this fact is at variance with results published by others, we undertook to obtain more direct evidence bearing on the metabolism of lignin.

The OCH_3, or methoxyl group, has been definitely shown to be present in lignin. The Kirpal and Bühn[2] modification of the Zeisel method was employed by us for the quantitative estimation of the methoxyl group.

Knowing the methoxyl content of the lignin and also that of the feces before and after the feeding of lignin, it was possible to follow up the demethoxylation in the animal organism. Experiments conducted with a dog showed that over 15% of the methoxyl initially present in the lignin was liberated and, in the case of the cow, 37% loss of methoxyl was observed. We believe that in these experiments we have direct evidence that lignin is metabolized in the animal organism.

We have also conducted experiments *in vitro* in which known quantities of lignin and fresh material taken from the 4 compartments of the cow's stomach were incubated at 38° C. for 8 days. It was found that demethoxylation took place in all our experiments, irrespective of whether or not toluene had been added to the medium. Experiments conducted in a similar manner, using fresh material taken from the large and small intestine of a cow gave entirely negative results. It would appear then that the demethoxylation of the lignin takes place in the stomach of the animal and that this is not brought about by bacteria, but rather by some other agent, possibly in the nature of an enzyme which is present in the gastric juice of the animal.

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[1] Csonka, F. A., Phillips, M., and Jones, D. B., *Proc. Am. Soc. Biol. Chem.*, 1928, vii, 24.

[2] Kirpal, A., and Bühn, T., *Ber.*, 1914, xlvii, 1084.

In connection with our animal experiments, we observed that when lignin was fed in larger doses than 2.0 gm. per kilo weight, toxic symptoms were developed. In 2 instances we found increased amounts of non-protein nitrogen in the blood, pointing to an impaired renal function.

4279

The Possible Significance of d-Xyloketose (Urine Pentose) in Normal Metabolism.

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The author[1] has pointed out that pentosuria is hardly likely to be a disorder of carbohydrate metabolism. The amount excreted is always small and is, apparently, unaffected by the administration of large quantities of glucose or galactose. Since the excretion of pentose is diminished in fasting,[2] it would seem that the most likely source is protein. The pentose may be the result of an abnormal metabolic sequence or an intermediate in normal metabolism, which is not further oxidized.

The experiment reported in this paper was designed to throw some light upon this question. It is intended to repeat and to extend the work in various directions; but circumstances preclude the immediate continuance of the work.

The *p*-bromphenylhydrazone of the pentose was prepared by a modification of the method employed by Levene and LaForge.[3] It melted at 127-8° and decomposed at 165°. In a 1% solution in alcohol, the rotation was −1.87° shortly after preparation and +2.43° after 24 hours. 7.20 gm. of this compound were decomposed with benzaldehyde, as described by Levene and La Forge. The filtrate from the benzaldehyde *p*-bromphenylhydrazone was extracted with ether and then evaporated, *in vacuo,* to 100 cc. In a 1 dm. tube, the rotation was 1.18°. Since the calculated yield of pentose was 3.39 gm, $[\alpha]_D^{22} = 34.8°$ The glucose equivalent of 1 gm. of pentose. calculated from the *p*-bromphenylhydrazone was 1.25 gm. by Sumner's method,[5] 1.45 gm. by the NaOH-picrate-acetone method[6] and 1.18 gm. by the Na_2CO_3-picrate method.[7]

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[1] Greenwald, I. In Endocrinology and Metabolism, ed. Barker, Hoskins and Mosenthal, New York and London, 1922, iv, 289.

[2] Klercker, K. O., *Deutsches Arch. f. klin. Med.*, 1912, cviii, 277.

[3] Levene, P. A., and LaForge, F. B., *J. Biol. Chem.*, 1914, xviii, 319.

This solution was injected into a young female dog, weighing 5.6 k., kept on a diet of 100 gm. beef heart, 16 gm. cracker meal, 16 cc. maize oil, 6 gm. bone-ash and 200 cc. water. There were 4 injections of 20 cc. and a fifth one of 10 cc., at intervals of approximately 2 hours. Calculated from the amount of hydrazone taken, the total amount of pentose injected was 3.05 gm. By Sumner's method, this showed a reducing action equivalent to that of 3.80 gm. glucose. The increase in the sugar content of the urine was equivalent to about 6% of the amount injected. A few days later, the animal received a similar series of injections of *l*-xylose. About 60% appeared in the urine. Another control experiment with glucose, gave, as was expected, no increase in the excretion of sugar.

d-Xyloketose is, therefore, not a substance that cannot be further metabolized by the organism. Whether or not it is a product of normal metabolism and what its action in insulin hypoglycemia, phlorhizin diabetes, etc., may be, are yet to be determined.

4280

Influence of Whole Wheat upon Hemoglobin Regeneration in Albino Rats.

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From the Nutrition Laboratory and the Institute of Child Welfare Research, Teachers College, Columbia University.

Young rats from mothers on a uniform diet (Sherman's Diet 13, consisting of one-third whole milk powder and two-thirds ground whole wheat plus common salt equal to 2% of the weight of the wheat with meat and lettuce 3 times a week) have been made anemic by exclusive feeding of fluid milk (pasteurized) from time of wean-

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[4] Sumner, J. B., *J. Biol. Chem.*, 1925, lxv, 395.

[5] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

[6] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1921, xlviii, 51.

[7] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1918, xxxiv, 194.

ing. At first all the animals were weaned at the age of 4 weeks, but in order to hasten the development of anemia, weaning at 3 weeks is now being done and 2 of the animals reported in this paper were so weaned.

Hemoglobin determinations have been made at intervals of approximately one week except immediately after weaning, when an interval of about 2 weeks was allowed to give time for the anemia to develop. The Newcomer method with the Newcomer disk,[1] having an accuracy of 5%, has been used, all determinations being done by 2 independent workers taking samples at the same bleeding. These readings have all agreed within the limits of experimental error. All colorimetric readings have also been checked by both observers.

When repeated hemoglobin determinations showed that the percentage in the blood had fallen to approximately half the amount normal for the age, as determined by Williamson and Ets,[2] one-half of the animals were continued on the milk diet as controls while the others were fed daily 6 gm. of whole wheat containing 0.2 mgm. of iron as determined by the Zimmermann-Reinhardt method[3] by 2 independent analysts. The animals ate the wheat well and were also supplied with fluid milk *ad libitum*.

The control rats, fed milk exclusively, have shown progressive lowering of the blood hemoglobin, in 11 weeks falling to values between 5.8 and 4.5 gm. per 100 cc. of blood.

The wheat-fed rats were tested weekly after the wheat feeding had been initiated. Starting with values ranging from 4.7 to 7.5 gm. of hemoglobin per 100 cc. of blood, there was a sharp rise, usually by the end of the first week, followed by a more gradual increase. Feeding was continued until the hemoglobin of the blood had risen to a figure above normal for the age and had remained high for several consecutive weekly readings. Values then ranged between 15 and 19 gm. per 100 cc. of blood. In no case have we failed to get a return to a figure above normal, whereas all the controls have continued to decline.

This is a part of a study of the place of whole-grain cereals in the diets of normal children.

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[1] Myers, Victor C., "Practical Chemical Analysis of Blood," 2nd Edition, 1924, 167.

[2] Williamson, C. S., and Ets, H. N., *Am. J. Physiol.*, 1926, lxxvii, 480.

[3] Fales, H. A., "Inorganic Quantitative Analysis," 1925, 330.

4281

Effect of an Exclusive Meat Diet on Chemical Constituents of the Blood.

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This is a preliminary report of an experiment to study the effect on human beings of an exclusive meat diet of several months duration. Our subjects were 2 Arctic explorers who had spent many years in the Arctic Circle, and while there, lived for the greater part of the time on a practically 100% meat and fat diet. Preliminary to our observations they were given careful physical surveys. Both were in excellent condition and showed no evidence of impaired health. Following these examinations studies were made of the respiratory exchange, ketogenesis, protein balance, mineral metabolism, fecal bacteriology, hematology and the chemistry of the blood. This report confines itself to the blood chemical findings.

Both men have eaten absolutely nothing but meat, cooked or raw Two experienced dieticians prepared and served the meat. No subjective untoward effects were noted.* Each subject consumed 120 to 130 gm. of protein and enough fat to total a daily caloric intake of 2200 to 2800. Both men were up and about, and took their exercise in walking. At times when they left the hospital they were accompanied by an attendant so that there might be no criticism as to the supervision of their diet. After 4 months the intensive metabolic studies were temporarily suspended. These subjects reported only once a month for blood chemical studies which we have continued for the past 11 months.

This communication deals with 2 questions: (1) Does an exclusive meat diet over a period of 11 months affect the kidney? (2) What changes, if any, are found in the blood of men receiving such a diet over the period mentioned? The following were studied: N. P. N. urea, uric acid, creatinine, NaCl, sugar, CO_2 C. P., serum Ca, plasma P, albumin (plasma), globulins, total protein (plasma), A/G, and cholesterol, before the meat diet and after an exclusive meat diet for 8 months. All analyses were done in duplicate whenever there was a question as to the correctness of the procedures analyses were checked at frequent intervals.

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* One of the subjects at one time developed nausea and diarrhea while on a lean meat diet; the other suffered from an incidental attack of influenza of 3 or 4 days duration. His recovery was uneventful.

The data may be summarized as follows: (1) 2 healthy men living exclusively on meat for the past 11 months, felt no untoward effects, maintained their weight and were in excellent health. (2) We find no evidence of renal impairment. (3) The chemical composition of the blood is little affected, except for a slight increase in uric acid and a temporary lipemia. The latter occurred significantly and only after unusual amounts of fat were taken.

4282

The Male Hormone.

CASIMIR FUNK AND BENJAMIN HARROW.

*From the Research Laboratory, Gaston Grémy, Paris, and the Department of Chemistry, College of the City of New York.**

The stumbling block in the successful demonstration of the testicular hormone has been the absence of a reliable animal test. Pézard and his collaborators found that caponized cockerels are good objects for demonstrating the presence of the hormone. They have shown that when ovarian grafts are introduced into such animals, the combs (and whiskers) diminish in size, become pale, and the feathers change in the direction of the female type. Testicular grafts, on the other hand, emphasize masculinity. Carindroit and Pézard[1] succeeded in demonstrating the presence of the male hormone in the blood of cocks. Busquet,[2] using the same test, concluded that the hormone exists in the blood of various young animals. He also claims to have obtained encouraging results by oral administration.

In view of the apparent successful demonstration of the presence of the male hormone in the circulating blood, and of the estrus-producing hormone in the urine of pregnant women[3] it seemed of value to investigate the urine of young men, in the hope of showing the presence of the male hormone.

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* This work has been made possible through grants obtained from Mr. A. Heckscher and Mr. Harold McCormick. Our thanks are also due to Dr. Henri-Rene Olivier for operating on the animals, and to Mr. A. Lejwa for technical assistance.

[1] Carindroit and Pézard, *C. r. soc. de biol.*, 1926, xcv, 296.

[2] Busquet, *C. r. soc. biol.*, 1927, xcvii, 1463.

[3] Lowe and Lange, *Klin. Wochenschr.*, 1926, v, 1038; Zondek, *Ibid.*, 1928, vii, 485; Veler and Doisy, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 806. The authors and Dr. Olivier can also confirm this work.

Lowe and Voss[4] show the presence of "testiculin" in the urine of men. The method, however, is not described, and no details are given. Oslund[5] records the injection of fresh sperm from the *vas deferens* into 3 caponized cockerels, resulting in positive comb growth. We present our results which, though still in its initial stages, is already based on considerably more experiments than those recorded by Oslund.

So far some 58 cocks, about 2 months in age, have been castrated. Of these, 13 died. Of the remaining 45, we believe the earlier 15 were badly castrated; but the later 30 seem to have been well castrated, judging by the results obtained.

Our source of hormone (or hormones?) was urine obtained from young men. The extract used was an evaporated filtrate obtained from an alcoholic precipitation. The extract was injected into castrated cocks and the length of comb measured each week. Control animals consisted of (a) non-castrated cocks and (b) castrated cocks. The results may be summarized as follows:

I. Six castrated animals. Daily urinary injections for 7 weeks. Increase in size of comb 30.5%. The injections were now stopped for 2 weeks and the increase was reduced to 15.5%. The injections were next resumed for 2 weeks, and the increase reached 28.0%.

II. Six non-castrated animals (controls), kept for 12 weeks. Increase 32.0%.

III. Nineteen castrated animals (controls), kept for 12 weeks. Increase 8.0%.

The experiments with the urine of young men were repeated a number of times, periods of 2-week injections alternating with periods of 2-week non-injections. In every instance injections caused the masculinising effect, and the rest periods gave rise to a shrinkage in the size of the comb.

We are inclined to the view that the urine of young men contains a substance (or hormones?) which has an activating influence on the comb growth of the animal.

Six of the castrated animals received daily urinary injections for 2 weeks (the urine representing that obtained from men 70-80 years old). The percentage *decrease* in comb growth was 9.0.

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[4] Lowe and Voss, *Klin. Wochenschr.*, 1928, vii, 1376.

[5] Oslund, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 845.

4283

The Sensitizing Dose in Respiratory Anaphylaxis (Asthma)*

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From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York University.

In our early studies on respiratory anaphylaxis[1] it was shown that guinea pigs could be sensitized by the inhalation of a dry antigenic dust. The animals were placed in a properly ventilated glass cage on a false wire floor. Dry horse dander was spread on the true floor of the cage. A nozzle, inserted through a hole near the bottom, was attached to a pump which blew in intermittent puffs of air, throwing the dust into a fine cloud. The air in the cage was kept moderately filled with the dust. The animals were exposed for 1 to 2 hours daily over a period of 6 to 12 days.

In our initial experiments[1] we were primarily interested in demonstrating that animals could be sensitized with a dry dust in a manner simulating the natural contact of a human being. Having demonstrated this, we desired to determine how long a period of exposure was necessary to sensitize animals by this route.

Two series of experiments were carried out in which the animals (300 to 400 gm.) were exposed in the above manner for varying intervals of time. Hypersensitiveness was proven by an intravenous injection (0.3 to 0.5 cc.) of horse dander extract after an incubation period of about 3 weeks.

In our first series we had 22 animals. After intravenous injection, of the 4 that were exposed for 1 hour, 2 were negative, 1 presented marked dyspnea with recovery and 1 presented marked dyspnea, suffusion of the eyes, collapse and recovery; the 5 exposed for 2 hours were all negative; the 3 exposed for 3 hours were all negative; of the 5 exposed for 4 hours, 1 was negative, 1 showed suggestive symptoms of anaphylaxis and 3 died in typical anaphylactic shock; and of 5 exposed for 5 hours, 4 died in typical anaphylactic shock and 1 showed marked anaphylaxis, convulsive movements, dyspnea and collapse with recovery.

With this group therefore it was only those animals which received 4 to 5 hours of contact with the dust that were sufficiently sensitized to die in anaphylactic shock, although we have an indica-

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* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 17; *Am. J. Dis. Child.*, 1927, xxxiv, 23.

tion that even after 1 hour's exposure there were moderate signs of sensitization.

In view of the evidence that animals might become sensitized after 1 hour's exposure, we carried out another series of experiments on 37 animals. After intravenous injection, of 4 animals exposed for ¼ hour, 1 showed suggestive symptoms, 3 were negative; of 7 exposed for ½ hour, 4 showed moderate dyspnea, and 3 were negative; 5 exposed for 1 hour all died a typical anaphylactic death; of 4 exposed for 2 hours, 1 was negative and 3 died in typical anaphylaxis; of 9 animals exposed for 3 hours, 7 died in typical anaphylactic shock, 1 showed dyspnea, suffusion of the eyes, moderate collapse with recovery and 1 was negative; of 5 animals exposed for 4 hours, 4 died and 1 showed moderate anaphylaxis with recovery; all 3 animals exposed for 5 hours died in typical anaphylactic shock.

Therefore a suggestion of sensitiveness may be brought about by the inhalation method in as short an exposure as ¼ hour. Definite sensitization may be established by 1 hour's exposure in certain animals. Other animals apparently cannot be sensitized at all.

4284

Effect of Hydroquinone in Vitamin A Deficiency.

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Since the discovery about 1913 that a lack of vitamin A in the diet would cause ophthalmia in rats, attempts have been made to show that this pathological condition might also be due to other factors than the absence of this vitamin. The so-called "salt ophthalmia" of McCullom, Simmonds and Becker[1, 2] has been shown by McCullom, Simmonds and Becker[3] and Jones[4] to be due to an oxidative destruction of vitamin A in the diet by ferrous sulfate if the

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[1] McCullom, E. V., Simmonds, N., and Becker, J. E., *J. Biol. Chem.*, 1922, liii, 313.

[2] McCullom, E. V., Simmonds, N., and Becker, J. E., *J. Biol. Chem.*, 1925, lxiv, 161.

[3] McCullom, E. V., Simmonds, N., and Becker, J. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 952.

[4] Jones, J. H., *J. Biol. Chem.*, 1927, lxxv, 139.

diets are not made up frequently. However, when the diet is made up daily, or every few days, no ophthalmia develops.

Recently Huston and Lightbody[5] have presented evidence to show that hydroquinone had a definite beneficial effect when used in a diet low or lacking in vitamin A. Huston, Lightbody and Ball[6] also concluded that hydroquinone had a definite anti-oxygenic effect on the vitamin A of milk fat and cod liver oil. If these views are correct, a very important physiological rôle must be attributed to this phenol.

In order to test out the hypothesis of Huston and Lightbody, we fed 2 groups of rats on a vitamin A free diet consisting of extracted and heated casein, 18 gm., corn starch, 51 gm., crisco, 23 gm., salt mixture (Osborne and Mendel), 5 gm., dried yeast, 3 gm., with 2 drops daily of a 5% solution of Vigantol to supply vitamin D. 0.05 and 0.1 gm. of hydroquinone per 100 gm. of the above diet were fed to the 2 groups of animals from the time of weaning. This diet was made up weekly. The 11 animals of these 2 groups all lost weight, developed ophthalmia and died.

When 1 part of a substance is included in 1000 parts of food the chances are very good that each animal will not receive a given amount of this small part each day. Hence a third group of rats were fed the above diet from the time of weaning and were given daily by mouth 5 drops of a solution of hydroquinone equivalent to 7 mg. of the phenol. The same results were obtained as described above. 3 animals of this group were cured of ophthalmia by cod liver oil.

In a study of the production of ophthalmia in mice we also gave hydroquinone without any beneficial effect. A control group of young mice were fed the following diet: edestin, 30 gm. corn starch, 63 gm., salt mixture (Osborne and Mendel), 7 gm., a 200 mg. yeast tablet and 1 drop of 5% Vigantol daily to supply vitamins B and D, respectively. The experimental group received this diet with the addition of 0.1 gm. hydroquinone per 100 gm. of food, from the time of weaning. Both groups developed ophthalmia and died from vitamin A deficiency in about 50 days.

From the above results we conclude that hydroquinone, when fed in the amounts stated above, has no beneficial effect in rats and mice suffering from a lack of vitamin A.

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[5] Huston, R. C., and Lightbody, H. D., *J. Biol. Chem.*, 1928, lxxvi, 547.

[6] Huston, R. C., Lightbody, H. D., and Ball, C. D., Jr., *J. Biol. Chem.*, 1928, lxxix, 507.

4285

Effect of Methyl and Ethyl Alcohol Mixtures on Behaviour of Rats in a Maze.

DAVID I. MACHT AND HARRIET LEACH.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Baltimore.

The pharmacological action of 2 or more drugs given in combination with each other is assuming great importance in recent pharmacological and toxicological research. The authors have been engaged in studying the pharmacological and toxicological effects of ethyl and methyl alcohol mixtures in various biological experiments, and one phase of the work is reported here.

The narcotic effects of ethyl and methyl, individually and in combination with each other, were studied on the behavior of the white rat in the circular maze. A total of 10 adult rats were used in the investigation, in addition to 6 rats which were used as controls. The rats were trained to solve the maze problem so that they could find their way from the periphery of the maze to its center, where food was placed, in the shortest period of time and with the least number of errors.

After the animals were thus trained, injections of weak alcohol solutions, 2 to 4%, were given repeatedly in doses of 2 cc. intraperitoneally and the effect on the running time and the number of errors noted. Several hundred such experiments were performed and the results obtained were summarized and averaged. The subjoined table expresses succinctly the effects produced. In the column marked "Running Time," the ratio of the running time after the injections of the various drugs is expressed in terms of per cent of the normal running time of the animals before injecting the drugs. In the column giving the number of errors the same procedure is employed, namely, the number of errors after injections of the alcohol are expressed as percentages of the normal number of errors of the rats before injecting the drugs.

It will be noted that methyl alcohol (chemically pure) was less depressant than the ethyl alcohol and, indeed, showed a definite stimulating effect. Ethyl alcohol was definitely depressant, though not to a very great degree. The most interesting findings were those obtained with mixtures of ethanol and methanol in equal proportions. Injections of such mixtures, containing only half as much of either constituent as were used in the experiments with individual alcohols, produced a very marked depression. Another

TABLE I. *Effect on Rats in Circular Maze.*

Drug Used	Method of Administration	Running Time Expressed as % of Normal	No. of errors made as compared with Normal
	Intraperitoneal Inj.	%	%
Ethyl alcohol	2 cc. 4% sol.	108	105
Methyl alcohol	2 cc. 4% sol.	87	85
Ethanol-methanol mixture (1:1)	2 cc. 4% sol.	122	120

series of experiments in which the alcohols were studied in regard to their effect on *learning* the maze problem, also indicated that a mixture of ethanol and methanol in equal proportions was more toxic than a double dose of either one. A similar synergistic effect of ethanol-methanol mixtures was noted by the authors in other toxicological experiments, which will be reported elsewhere. The present findings are of obvious practical interest.

4286

Production of Monocytes and Epithelioid Cells in Subcutaneous Tissues by Injection of Various Irritants.*

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From the Departments of Medicine and Anatomy, Vanderbilt University Medical School.

In the course of a study upon hepatic injury and the possible relationship of such injury to the white blood cells, Lawrence and Huffman[1, 2, 3] found that the subcutaneous injection of guinea pigs with yellow phosphorus (in oil) was followed by an elevation of the monocytes in the circulating blood. These findings suggested that the study of the subcutaneous tissues of animals injected with the same material might aid in determining the cause and site of the production of the monocytes.

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* This work has been aided by a grant from the Research Committee of the National Tuberculosis Association.

[1] Huffman, M. M., Lawrence, John S., and Jones, Edgar. The effect on the white blood cells produced by the intraperitoneal injection of whole liver. In press.

[2] Lawrence, John S., and Huffman, M. M. An increase in monocytes in the blood following the subcutaneous administration of yellow phosphorus in oil. In press.

[3] Lawrence, John S., and Huffman, M. M. Fatty changes in the Kupffer cells of the liver of the guinea pig in phosphorus poisoning. In press.

It has been repeatedly demonstrated that the injection of many supposedly inert substances (*e. g.*, waxes, oils and various colloidal bodies) is followed by local reactions which are similar to those seen in fixed sections of tuberculous tissues. With the methods involving fixation and sectioning, it has been possible to locate groups of epithelioid cells in the tissues; but these methods have not given us any very satisfactory help in identifying epithelioid cells when occurring singly or scattered in the tissues. The use of the supravital method of staining[4, 5] has added certain criteria to those already known and has made possible the recognition of the epithelioid type of cell (including the epithelioid cell of tuberculosis) even when seen entirely alone in the connective tissues. The principal factor in this characteristic appearance is the presence of many fine droplets in the cytoplasm (granules or vacuoles) which stain with neutral red, and the later appearance of refractive droplets in the periphery of the cell.[6] The development of these characteristic cells (epithelioid) in tuberculous areas was demonstrated by Cunningham, Sabin, Sugiyama and Kindwall, but these observers did not pursue their studies into an analysis of the nature of the basic stimulus of this production or modification, or into the possible production of monocytes and epithelioid cells under other abnormal conditions.

The present studies have been made with the supra-vital technique referred to above, both neutral red and Janus green being used. Guinea pigs have been used throughout and all animals have been carefully autopsied at the end of the experiment to rule out intercurrent infection. Phosphorus dissolved in almond oil, olive oil, or a mixture of both; almond and olive oils alone; and mineral oil have been used for the subcutaneous injections. Very small amounts were given in the subcutaneous tissues of the anterior abdominal wall, the area was carefully marked and the animal kept for various intervals before final study. At the time of study, the animal was killed with chloroform and the area of the injection exposed. Scrapings were made and studied immediately on supra-vital slides.

The smears made in this way invariably revealed masses of monocytes which varied from the younger forms with very little neutral red, to large cells containing large numbers of vacuoles filled with the dye and often containing refractive droplets (probably lipoid)

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[4] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1921, xxxii, 314.

[5] Simpson, M. E., *Anat. Rec.*, 1921, xxi, 82.

[6] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 231.

in their periphery. They have likewise always revealed typical epithelioid cells with the characteristic arrangement of neutral red. These epithelioid cells have at times progressed to the stage of degeneration which is seen so commonly in tuberculosis and which is indicated by the decrease in the amount of the neutral red and by the accumulation of large numbers of refractive droplets. From time to time a moderate reaction on the part of the granulocytes has also been evident and typical clasmatocytes have been present, but always the striking observation has been the massive number of monocytes in all stages of development, modification and degeneration into epithelioid cells.

The element that time plays in these various changes, the determination of the variety of substances which are capable of producing these changes, the question as to whether such substances have a chemical similarity or only one of a physical nature (*i. e.*, in this specific action) are still being investigated. In brief, the essential aim of these studies is the determination of the character of the stimulus which brings about an increase in monocytes and the formation of epithelioid cells in disease processes.

4287

Production of Epithelioid Cells in Lymph Glands by Injection of Non-Tuberculous Substances.

DUNCAN C. HETHERINGTON. (Introduced by R. S. Cunningham.)

From the Department of Anatomy, Vanderbilt University Medical School.

Until the introduction of certain dyes permitting the staining of cells in the living condition, the epithelioid cell was a rather vague cytological entity. However, it is now possible to define it morphologically (as seen supra-vitally stained with neutral red) as a relatively large cell having a great number of very fine granules or vacuoles, arranged in a rosette, which assume with this vital dye a salmon pink to a brownish red tint. Such an appearance furnishes neither definite nor conclusive evidence regarding the origin or function of this cell, but does, on the other hand, very clearly define it as a cell of distinctive characteristics. This specific characteristic staining quality was first noted by Sabin (1923) in cells obtained from tuberculous lymph nodes, and similar cells have since been observed by other investigators in tuberculous tissues generally. As it had been found that epithelioid cells are very numerous in tuberculous involvement of the lymph nodes, it seemed that these structures would be a favorable location for the study of the possible effect of non-tuberculous substances.

Rabbits were used for these experiments. They were subjected to laparotomy and the mesenteric lymph nodes were exposed. By means of small glass pipettes, material was removed from various parts of the nodes, the experimental materials were then injected into the substance of the nodes, and the incision closed. The substance used in these experiments have been sterile macerated rabbits' brain, olive oil (with and without phosphorous), cod liver oil (unphosphorated), and mineral oil. All of the aspirated material was studied on supra-vitally stained slides.

In the material from the normal nodes, a few monocytes were usually, but not invariably, found, but in none of them were any epithelioid cells seen.* The animals were sacrificed 72-120 hours after the injection of the experimental material and many supravital preparations were carefully studied. In the rabbits which had been given macerated brain, examination brought to light an abundance of typical epithelioid cells and a large number of monocytes. A similar but not as marked production of monocytes and epithelioid cells was found in the animals injected with the other substances mentioned above.

It is impossible at the present time to fully evaluate these observations. Since Cunningham and Sabin[1] believe that the epithelioid cell may be a transformed or modified monocyte, and since Lewis and Lewis[2] have described the formation of epithelioid cells from monocytes, attention must be called to the coincidental increase in the monocytes and epithelioid cells in these experiments. Regardless of any interpretation which it may be possible to put upon these observations, it seems wholly clear that typical epithelioid cells (indistinguishable by the methods used from those found in tuberculous nodes) can be produced in lymph nodes *in vivo* by factors other than an active infection by the tubercle bacillus.

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* It is important to note that a large number of normal mesenteric lymph nodes have been studied both by the puncture method and by scraping the cut surface of the node and in none of these have typical epithelioid cells been found.

[1] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 231.

[2] Lewis, M. R., and Lewis, W. H., *J. Am. Med. Assn.*, 1925, lxxxiv, 798.

4288

Cannibalism in a Ciliate, Blepharisma.

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From the Zoological Laboratory, Harvard University.

In mass cultures of several different races of *Blepharisma undulans,* including one which has been cultured by the writer for several years and used in connection with other investigations, cannibalism has occurred frequently. This phenomenon has not been very generally noted among ciliate protozoans, as the writer found in reporting a similar case in the Hypotrichida,[1] and there are certain aspects of this process in Blepharisma which promise to add to the knowledge of digestive processes in ciliate protozoa.

Cannibalism takes place in mass cultures in which growth has been rapid and large numbers of Blepharisma are present. The animal about to become a cannibal is always considerably larger than the individual that is ingested. The process of ingestion is relatively slow, requiring several minutes for completion. Ingested animals are not readily killed by the cannibal but show movements for varying periods, in one case as high as 8 hours. Ingestion is not always followed by digestion of ingested individuals; not infrequently the ingested animal is ejected and numbers of these have been cultured successfully after this occurrence. Once the ingested animal is killed digestion takes place at room temperature usually within about 24 hours.

Of special interest in relation to this phenomenon is the fact that Blepharisma contains a pigment which under certain conditions is red. There is considerable evidence to show that this pigment is a natural indicator. The process of digestion is accompanied by color changes in this pigment and investigation of the significance of these changes is now in progress.

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[1] Dawson, J. A., *J. Exp. Zool.*, 1919, xxix, 473.

4289

Cholesterol in Blood of Horseshoe Crab and Woolly Bear Caterpillar.

RICHARD E. SHOPE. (Introduced by Paul A. Lewis.)

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It is generally considered that the blood of all animals contains both free cholesterol and cholesterol esters, the former existing in corpuscles and plasma and the latter occurring only in the plasma or serum. Since this conception is founded upon the results of determinations carried out on the blood of higher animals it seemed of interest to determine whether lower and more primitive forms would also contain these constituents in their blood corpuscles and serum.

The horsehoe crab *(Limulus polyphemus)* and the woolly bear caterpillar *(Isia isabella)* were chosen as suitable experimental animals both as to position in the animal scale and as to the ease with which sufficient blood could be obtained. The work on the horseshoe crab was carried out on 2 adult animals, and that on the caterpillars on pooled blood samples from 40 animals. Bloor's[1] method was used in determining total cholesterol and Bloor and Knudson's[2] in determining cholesterol ester, both modified as to amount of material tested and amount of extractive used in anticipation of very low values. The results obtained are given in Tables I and II.

Summary. The blood serum of *Limulus polyphemus* was found to be entirely free of cholesterol or cholesterol esters and thus differs

TABLE I. *Horsehoe Crab (Limulus polyphemus).*

Animal No.	Total cholesterol in blood cells.	Cholesterol ester in blood cells.	Total cholesterol in blood serum.	Cholesterol ester in blood serum.
	mg. %	mg. %	mg. %	mg. %
1	67.2	0	0	0
2	75.6	0	0	0

TABLE II. *Woolly Bear Caterpillar (Isia isabella).*

Total cholesterol in blood cells.	Cholesterol ester in blood cells.	Total cholesterol in blood serum.	Cholesterol ester in blood serum.
mg. %	mg. %	mg. %	mg. %
240.0	0	27.7	0

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[1] Bloor, W. R., *J. Biol. Chem.*, 1916, xxiv, 227.

[2] Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, xxvii, 107.

from the blood sera of higher animals. The blood cells, however, contained free cholesterol and were similar to blood cells of higher animals in that they contained no cholesterol esters. Both blood cells and serum of the woolly bear caterpillar contained free cholesterol but no cholesterol ester was present.

4290

Comparison of the Resistance of Streptococci and of Poliomyelitic Virus in Glycerol.

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The virus of poliomyelitis, present in monkey brain tissue has been shown to be virulent after being kept 8 years in 50% glycerol at 4° C.[1] Micrococci have been obtained in culture from the "glycerolated nervous system of cases of poliomyelitis after a period of 15 months."[2]

Streptococci and other microorganisms were isolated from the brains of monkeys recently infected with the virus of poliomyelitis and from those of normal monkeys.[3]

Rabbits were inoculated intracerebrally with certain of the streptococci.[3] The brains of these rabbits were removed under aseptic precautions and were stored at 4° C. in 50% glycerol. At frequent intervals cultures were made by placing a small fragment of washed glycerolated brain material in dextrose broth. Seven brain specimens were thus tested. One brain inoculated with streptococci yielded a pure culture of the organisms after 82 days, but not after 116 days; one after 116 but not after 146 days, and three after 117 but not after 204 days. In 2 of the brains tested streptococci were still viable after 303, but not after 333 days.

It is evident that while streptococci can survive in brain tissue in 50% glycerol for periods up to nearly a year, there is wide disparity between the resistance of the virus of poliomyelitis and the resistance of certain strains of streptococci in glycerol.

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[1] Rhoads, C. P., *J. Exp. Med.*, in press.

[2] Mathers, G., and Weaver, G. H., *J. Infect. Dis.*, 1918, xxii, 559.

[3] Long, P. H., Olitsky, P. K., and Stewart, F. W., *J. Exp. Med.*, 1928, xlviii, 431.

4291

Spermatogenesis Following Early Ovariotomy in the Brown Leghorn Fowl.*

L. V. DOMM. (Introduced by F. R. Lillie.)

From Whitman Laboratory of Experimental Zoology, The University of Chicago.

In our earlier series of experiments on ovariotomy[1] operations were performed at a relatively late age, the majority of the birds ranging from 3 to 8 or 9 months. One of the extraordinary results revealed was the development of the rudimentary right gonad into an organ which is essentially a testis though, in our cases, always sterile. This was particularly baffling since during the course of our study 2 cases of spermatogenesis were recorded by Benoit[2] and 1 by Zawadowsky[3] in the ovariotomized fowl, from a relatively insignificant number of experiments. These birds were 4, 27, and 45 days of age respectively at the time of operation. Only 2 of our cases, one of 2, and one of 27 days, came within this age range.

Furthermore, according to the work of Brode[4] and others[5, 6] primordial germ cells are present in the medulla of both right and left gonads of the female chick in early stages. Brode has shown that these cells gradually decrease in numbers after hatching so that by approximately 3 weeks after they have practically all disappeared in the right gonad. Since it is highly probable from these observations that no primordial germ cells were present in the right rudimentary gonad at the time of ovariotomy in our earlier series it was considered advisable to supplement this otherwise extensive data by additional cases of early ovariotomy.

A large number of females were ovariotomized at an early age. Operations were performed every day from the first to the 30th day, and every other day from the 30th to the 50th day, after hatching. 175 birds operated under 30 days of age survived the operation; of these, 26 had been operated during the first 7 days.

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* This investigation was supported in part by a grant from the Committee for Research in problems of sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 28; *J. Exp. Zool.*, 1927, xlviii, 31.

[2] Benoit, Jacques, *C. R. Acad. Sc.*, 1923, T. 177, 1074.

[3] Zawadowsky, M., *Trans. Lab. Exp. Biol.*, Zoopark, Moscow, 1926, ii, 164.

[4] Brode, M. D., *J. Morph. and Physiol.*, 1928, i, 1.

[5] Swift, C. H., *Am. J. Anat.*, 1914, xv, 483; *Am. J. Anat.*, 1915, xviii, 441.

[6] Firket, Jean, *Arch. de Biol.*, 1914, xxix, 201; *Arch. de Biol.*, 1920, xxx, 393.

The birds were killed at regular intervals and the material carefully preserved. The study is not complete. Forty birds are still alive, and the gonads of those killed have not all been studied histologically. The experiments, as previously, were confined to the single comb light brown Leghorn secured from the same source immediately after hatching.

This supplementary series should give us a definite understanding of the histogenesis of this testis-like right gonad about which such varying views have been expressed. It was hoped that it would definitely reveal whether it had the capacity to develop spermatogenetic tissues following ovariotomy. We have now studied the histological preparations from 72 such testis-like right gonads of the second series and in 5 of these we find spermatogenesis.

Bird No. 1038. This bird was ovariotomized when 7 days old and killed 8 months after the operation. Following the operation it developed the plumage and head furnishings of the male. During the 2 months prior to its death the new plumage was intermediate in type. Post mortem examination revealed a relatively prominent right gonad measuring 2.0 cm. in length and 0.9 cm. in width. The entire gonad was serially sectioned. Active spermatogenesis appears in a few of the tubules on the 34th slide of the series and runs through to the 40th. These tubules are thus confined to a rather limited area of the gonad, the rest of which reveals a scattering of the characteristic sterile tubules previously found.

Bird No. 1083. This bird was operated when 8 days old and killed 11 months following the operation. By 6 months after the operation the bird was completely male plumaged, no other plumage was developed. The head furnishings developed very slowly and did not attain male proportions. Post mortem examination revealed a small right gonad measuring 1.8 cm. in length and 0.5 cm. in width. Only the anterior part of this gonad has been sectioned. It shows spermatogenetic tubules, many with fully differentiated spermatozoa, closely packed throughout the entire section.

Bird No. 1008. This bird was ovariotomized when 11 days old and killed 8 months following the operation. It developed the plumage and head furnishings of the male. During the first 4 months it developed juvenile male plumage which was followed by adult male for but a short period. By 5 months intermediate plumage began to appear, followed by female at 7 months. Female plumage continued to appear up to the time of killing. Post mortem examination revealed a prominent right gonad measuring 2.4 cm. in length and 1.0 cm. at its widest point. The anterior third of this gonad has been sectioned covering 30 slides. Spermatic tubules appear toward the end of the series, being limited to about 9 slides. They are rather closely packed and confined to a limited area dorsally immediately under the albuginea. The rest of the section reveals a scattering of sterile tubules interspersed with connective tissue.

Bird No. 1002. This bird was ovariotomized when 17 days old and killed 11½ months later. Following the operation it developed the plumage and head furnishings of the male. The juvenile plumage of the male was developed following the operation which was later followed by adult male. By 10 months intermediate plumage appeared and when the bird was killed the ingrowing back and saddle feathers were female. Post mortem examination revealed a prominent right gonad measuring 2.4 cm. in length and 0.9 cm. at its widest part. The anterior third of this gonad has been sectioned, covering 30 slides. Here spermatic tubules, many of which reveal mature sperm, appear in the 5th slide and run through the rest of the sectioned series. In general these tubules are loosely scattered through the entire section, though in places they occur in small groups. Some of the characteristic sterile tubules are also found.

Bird No. 923. This bird was ovariotomized when 38 days old and killed 12 months later. The juvenile plumage of the male was developed following the operation and this was later replaced by adult male. Seven and one-half months after, intermediate plumage was appearing, and from 9½ months after, until the bird was killed the new plumage was female. Head furnishings attained male proportions. The bird crowed. Post mortem examination revealed a prominent right gonad measuring 2.1 cm. in length and 0.9 cm. at its widest point. The entire gonad was serially sectioned, covering 115 slides. Here spermatic tubules, showing mature sperm, do not appear prior to the 64th slide of the series, where a few are found along the periphery. They are found in the next 3 slides and then apparently disappear in a few slides, then reappear, running through to slide 101, where some of them seem to end in the characteristic sterile tubule.

In other cases of this series, not here described, the right gonad developed ovotestis, also perfectly typical ovary, which definitely reveals that the changes following complete ovariotomy are very variable. Including the cases here described we have now demonstrated a range of possibilities for the right gonad that extends all the way from complete spermatogenesis following ovariotomy, through sterile testis and ovotestis, to complete and normal ovogenesis associated with a corresponding array of other sex characters such as accessory organs of reproduction, head furnishings, behavior, plumage and spurs.

These results lead to the working hypothesis that primordial germ cells are necessary for gametogenesis, that when they become incorporated in the cords of the medulla they produce spermatogenesis and when in the cortical elements of the gonad they produce ovogenesis. This working hypothesis agrees with Witschi's[7] findings in Amphibia.

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[7] Witschi, Emil, Proc. Soc. Exp. Biol. and Med., 1928, xxv, 729.

Western New York Branch.

Cornell University Medical College, Ithaca, December 15, 1928.

4292

Conditioned Motor Responses in Children.

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(Introduced by H. S. Liddell.)

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Pavlov's researches[1] upon the activities of the cerebral hemispheres in the dog have inspired a number of workers to employ his method of the "conditioned reflex" in the study of human behavior. Outstanding among these workers are Krasnogorski and Ivanov-Smolensky. In most of his work with children Krasnogorski uses the motor response of the opening of the mouth to receive a bit of candy, forming associations between this response and various stimuli, *e. g.,* the beat of a metronome.[2] Ivanov-Smolensky uses the motor response of squeezing a rubber bulb which operates a shutter, the shutter allowing a piece of candy to fall into a tray from which the child recovers it.[3]

Using Ivanov-Smolensky's apparatus,[3] we have begun a study of the behavior of children by the method of conditioned responses. We have established in two 6 year old children conditioned motor responses to 3 different stimuli, the beat of a metronome, the sound of an ordinary buzzer and the flash of a small red light. Eight or 10 joint stimulations with the metronome (buzzer or light) and the candy were sufficient to establish a conditioned response. After the joint stimulations, when the metronome (buzzer or light) alone was presented, the child would squeeze the rubber bulb. A much larger number (200-300) of joint stimulations was required to establish a conditioned response to the metronome in a 4 year old child. The development of the conditioned response in the 4 year old was inhibited (Pavlov's "external inhibition") by her responses to various stimuli, objects in the laboratory at which she looked or nearby parts of the apparatus which she handled. No doubt, other factors would explain, in part, her failure to become conditioned sooner.

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[1] Pavlov, I. N., *Conditioned Reflexes,* 1927, Oxford University Press.

[2] Krasnogorski, N., *Am. J. Dis. Child.,* 1925, 753.

[3] Ivanov-Smolensky, A. G., *Brain,* 1927, L. 139.

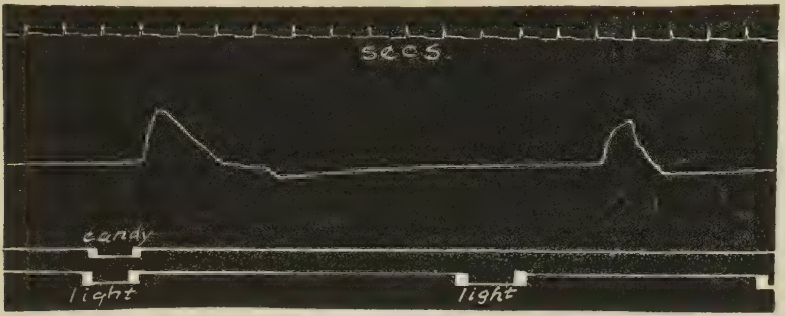

FIG. 1.

In the kymograph record (Fig. 1) the upper line is the time record in seconds, the line next below is the child's response. The third line from the top is the record of the signal lever for the candy stimulus, the fourth line is the record of the signal lever for the light stimulus to which the child is being conditioned. The response at the left of the figure (eighth or tenth joint stimulation) shows the light stimulus ("conditioned stimulus") accompanied ("reinforced") by the stimulus of the candy. The response at the right of the figure ("conditioned response") shows the child's reaction to the light stimulus ("conditioned stimulus") alone after 8 to 10 joint stimulations.

4293

Destruction of Botulinum Toxin by Bacillus subtilis.

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In previous papers[1, 2] it has been shown that a variety of bacteria have the ability to destroy the toxin produced by *Clostridium botulinum*. This power, though definite, is relatively slight as compared with the ability of certain organisms to destroy the toxin

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[1] Sherman, J. M., Stark, C. N., and Stark, Pauline, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 546.

[2] Sherman, J. M., Stark, C. N., and Stark, Pauline, *J. Bact.*, 1928, xv, 35.

of the diphtheria organism.[3] In our work on the destruction of the toxin of botulism there has not appeared to be any general relationship between the proteolytic activities of the organisms tested and their ability to destroy botulinum toxin. In fact, it has been found that such an active proteolytic organism as *Clostridium sporogenes* has little or no demonstrable capacity to destroy this toxin, under the conditions of our experiments.[4] On the other hand, Dack,[5] working with mice, showed a definite destruction of botulinum toxin by several strains of *Clostridium sporogenes* and also by a variety of other spore forming anaerobic organisms.

The purpose of this note is to report the results obtained with *Bacillus subtilis,* which shows a more marked destruction of botulinum toxin than does any one of the other organisms with which we have worked.

The toxin was made by growing *Clostridium botulinum,* type A, in a medium composed of: beef infusion, 4% peptone, 0.5% glucose, 0.7% di-basic potassium phosphate, and 0.3% mono-basic potassium phosphate. The culture was grown in this medium for 8 days at 37° C. It was then filtered and the sterility of the filtrate verified by cultural tests. Some of the filtrate was then inoculated with a laboratory culture of *Bacillus subtilis* and incubated for 14 days at 37° C. Other portions of the same filtrate uninoculated were similarly incubated as controls.

Destruction of Botulinum Toxin by *Bacillus subtilis*

	Approximate M. L. D. titer			
	10	25	300	350
Control			+	—
"			+	—
B. subtilis	+	—		
" "	+	—		

+ = Death of 250 gm. guinea pig in 4 days.
— = Survival of 250 gm. guinea pig.

As may be seen from the accompanying table, *Bacillus subtilis* reduced the toxin titer of the botulinum filtrate from about 300 M. L. D. to about 10 M. L. D. per cc. as determined by guinea pig inoculations.

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[3] Stark, C. N., Sherman, J. M., and Stark, Pauline, *J. Infect. Dis.*, in press.

[4] Stark, C. N., 1927, Thesis, Cornell University.

[5] Dack, Gail M., *J. Infect. Dis.*, 1926, xxxviii, 165.

4294

Influence of Epinephrine on Blood Sugar Utilization of Functionally Hepatectomized Rats.

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Previous experiments[1] have led to the conclusion that one of the principal causes for the epinephrine hyperglycemia consists in a decreased utilization of blood sugar in the tissues. It was pointed out that mobilization of liver glycogen alone is an inadequate explanation for the hyperglycemia, because there is not enough liver glycogen present to provide the tissues for hours with sugar at a rate which is greater than their normal ability to utilize sugar. Mobilization of liver glycogen can only lead to a protracted hyperglycemia of the type observed after epinephrine injections, (1) if a new formation of liver glycogen occurs which keeps pace with the conversion of liver glycogen into blood sugar and (2) if utilization of blood sugar itself is diminished. A new formation of liver glycogen exceeding its mobilization was demonstrated during epinephrine action and the source for the newly formed liver glycogen was found to be lactic acid derived from muscle glycogen.[1] That blood sugar utilization is low during epinephrine hyperglycemia was suggested by a comparison of the arterial and venous blood sugar concentration in men. Whereas the arteriovenous difference rises during alimentary hyperglycemia, it fails to do so during the much more pronounced epinephrine hyperglycemia, showing that the tissues are unable to withdraw sugar from the blood at a higher rate. A carbohydrate balance on sugar fed rats showed that the disposal of absorbed sugar after epinephrine injections is markedly diminished in the peripheral tissues.[1]

Since epinephrine inhibits blood sugar utilization in the peripheral tissues of the intact animal, this should also be demonstrable on hepatectomized animals. Mann[2] found that epinephrine did not cause an increase in the blood sugar following hepatectomy or delay the development of the characteristic symptoms associated with hypoglycemia. This is not surprising, because muscle glycogen cannot contribute glucose directly to the blood; it can only do so indirectly through the intervention of the liver. In the absence of the liver, lactic acid derived from muscle glycogen can no longer be converted into glucose and consequently epinephrine is unable to produce hyperglycemia in hepatectomized animals. Since it takes several hours until the blood sugar falls to 50 mg. % in the hepatectomized dog and since the time at which hypoglycemic symptoms appear is variable, an inhibitory action of epinephrine on the fall in blood sugar might easily escape attention, or it might even be absent, owing to the extremely low utilization of blood sugar. In order to demonstrate the inhibiting action of epinephrine in hepatectomized animals it is necessary to provide for a larger utilization of blood sugar by means of a glucose injection.

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[1] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, lxxix, 309, 321, 343.

[2] Mann, F. C., *Medicine*, 1927, vi, 419.

The experiments were made on rats fasted previously for 24 hours. Such animals contain 5.8±1.4 mg. liver glycogen per 100 gm. rat (average of 24 determinations). The tolerance limit for intravenously injected glucose is at a rate of 250 mg. per 100 gm. rat per hour.[3] When 0.03 mg. of epinephrine is injected subcutaneously and glucose supplied at a rate of only 100 mg. per 100 gm. rat per hour, an average of 75% of the amount of glucose injected remains unutilized in the blood and body fluids. This result on intact animals cannot be explained either by a failure of the liver to form glycogen or by mobilization of liver glycogen, because without epinephrine injection, only 10 to 15% of the 250 mg. of glucose utilized per hour is disposed of in the liver. The hyperglycemia must therefore be due to an inhibition of blood sugar utilization in the peripheral tissues. Since there was the possibility that the presence of the liver was essential in some other way for the action of epinephrine on blood sugar utilization, the above experiments were repeated on functionally hepatectomized rats. For this purpose the portal vein was subtotally ligated in a preliminary operation. Ten to 21 days later the hepatic pedicle was completely ligated under amytal anesthesia and glucose was infused at a constant rate into a femoral vein. In all cases the sugar and lactic acid content of the blood was higher after glucose plus epinephrine than after glucose alone (10 experiments each). The average values were as follows. *No epinephrine:* glucose infused per 100 gm. rat per hour 109 mg.; blood sugar 134 mg. %; blood lactic acid 55 mg. %. *Epinephrine:* glucose infused per 100 gm. rat per hour 110 mg.; blood sugar 246 mg. %; blood lactic acid 135 mg. %. The rather high blood lactic acid of the rats receiving glucose alone is due to the absence of the liver, since this organ normally removes lactic acid from the blood and the further increase in blood lactic acid after epinephrine is due to mobilization of muscle glycogen. When the hepatic pedicle was tied but no glucose injected, the rats developed the typical symptoms associated with hypoglycemia which could be relieved by the injection of glucose. The above experiments will be repeated on completely hepatectomized animals.

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[3] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1927, lxxii, 597.

4295

Absorption of Glucose From Alimentary Tract of Rats Deprived of Vitamin B Complex.

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(Introduced by J. R. Murlin.)

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Rats from 3 different colonies were placed upon adequate diets and diets deficient in the vitamin B complex. The absorption of glucose from the alimentary tract of these rats was determined by means of the method devised by Cori.[1]

The rate of absorption of glucose was not constant for the 1, 2, and 3-hour periods, there being a marked falling off during the last hour of a 3-hour period. This does not agree with Cori's findings.

The percentage of glucose absorbed during 1, 2, and 3-hour periods appeared to be dependent upon the amount of glucose remaining unabsorbed in the alimentary tract.

Two groups of animals which had been on a diet containing no vitamin B absorbed a smaller percentage of the glucose fed than did normal animals. A third group of animals, which had come from a colony whose members were able to resist the effects of vitamin B-deprivation, did not show a decreased absorption of glucose following a period during which they had received a diet containing no vitamin B.

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[1] Cori, C. F., *J. Biol. Chem.*, 1925, lxvi, 691.

4296

Ephedrine Homologs and Isomers: Relationship Between Their Pharmacological Action and Their Chemical Constitution and Stereoisomerism.*

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The recent wide clinical use of ephedrine has led a number of chemists to synthesize its homologs and isomers in the hope that some of them might possess the same physiological action and might eventually be used as substitutes for ephedrine. The present writers have studied and compared from the pharmacological side a series of 24 compounds, including 6 optical isomers of ephedrine. All these compounds may be considered as derivatives of β-phenylethylamine having the general formula $C_6H_5.CHH'.CHR.NR'R''$, where $H' = H$ or OH, and R, R$'$ and $R'' = H$ or alkyl radicals, respectively. The investigation included the pressor action in animals and in men, the mydriatic action in animals, the astringent property on the human nasal mucous membrane, the action on the isolated intestines and uterus and on the frog's heart, and the toxicity in rabbits. It was found that the primary amines are most, while the tertiary amines are least, active, the secondary amines being intermediate. The pressor action diminishes with the increase in the number of C-atoms in the alkyl groups R, R$'$ and R$''$, and in several instances is changed to a depressor action. The introduction of an OH group on the β-C-atom from the amino radical reduces the activity of the substance. The presence of a methyl, or ethyl group at R, when $R' = H$ or CH_3 and $R'' = H$, confers on that compound a more prolonged pressor action. The toxicity is higher in primary amines than in secondary and tertiary amines. It increases with the increase in the number of C-atoms in the alkyl groups R, R$'$ and R$''$. The introduction of an OH group on the β-C-atom reduces the toxicity. The depressant action on the frog's heart runs parallel to the toxicity. The majority of these compounds contract the congested nasal mucosa in men. Of the optical isomers of ephedrine, the *l*-form is 3 times as strong as the *d*-form and *d*-pseudoephedrine 7 times as strong as the *l*-pseudoephedrine as determined by their pressor action in pithed cats. Both *l*-ephedrine and *d*-pseudoephedrine occur in nature. The above results may offer a chemical explanation of the typical action of ephedrine, and suggest the synthesis of new compounds which may possess the clinical advantages of both ephedrine and epinephrine.

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